A Monograph on **Guar Gum** 10/17/72

A MONOGRAPH

on

GUAR GUM

to

Food and Drug Administration
Department of Health, Education and Welfare

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SUMMARY

Guar gum is the ground endosperm from the seeds of the Cyamopsis tetragonalobus (L) Taub plant.[41] It is a complex polymer containing many mannose and galactose units, or galactomannan, having a molecular weight in the range of 200,000 to 300,000.[39]

Guar gum hydrates rapidly in water to give highly viscous, non-Newtonian colloidal solutions which are stable over a wide pH range. Unless preserved, aqueous solutions of guar gum are susceptible to biodegradation.[78] As for other natural gums, general methods are available for the isolation and detection of guar gum in foods, although its infrared spectrum is not distinguishable from that of locust bean gum.[33,39,51,94] No suitable method has been developed for its quantitative determination in food products.

Because of its ease of dispersion in cold water, its excellent solubility and hydrocolloid properties, guar gum has found wide use in the food industry. Over 4,300,000 pounds were reported used in food products in 1970.[10]

When administered at a level of 27 percent of the diet, guar gum caused physical blockage of the intestinal tract in rats and led to death in 7 of 10 animals within one week.[116] No other signs of toxicity were found at necropsy. At a level of 17 percent of the diet for a one week period, guar gum had the same caloric value as cornstarch in rats and caused no toxic effects.[116] At 30 percent of the diet for 48 hours, guar gum had no overt toxic effects and contributed to the liver glycogen level of rats.[61]

Guar gum has also been fed to rats at 6 percent of diet for 91 days and 5 percent of diet for 6 months with no adverse effect on weight gain, hematology, organ weights or organ histology.[19,60]

In chickens, one study[62] has been reported where 2 percent guar gum in the diet for a period of 3 weeks had an adverse effect on weight gain, N retention, fat adsorption and pancreas weight but this has not been confirmed in other studies.[24,34]

Monkeys have been fed 1g/day (~200mg/kg/day) for 6 months with no apparent adverse effects, although the final results of this study are not available.[60]

While guar gum does appear to be adsorbed and utilized in the diet of rats,[61,116] further studies are needed to elucidate the metabolic fate of guar gum in man.

Existing data do not indicate a need for restricting the daily intake of this gum for man. However, long term animal feeding studies have yet to be performed.

CHEMICAL INFORMATION

Nomenclature

Common Names

Guar gum is the name of the natural gum obtained by milling the seeds of the guar plant, Cyamopsis tetragonalobus (L) Taub, or Cyamopsis proraloides, family Leguminosae[41,78]. Guar gum, obtained from grinding the endosperm portion of the seed, is sometimes referred to as guar flour.[113] By addition of alcohol to an aqueous solution of guar gum, a purified galactomannan polysaccharide is obtained which has been given the name guaran[78].

Chemical Names

Guar gum does not have a completely descriptive chemical name. In somewhat general terms it is a high molecular weight carbohydrate polymer made up of many mannose and galactose units, or galactomannan, linked together in the pattern shown in the structural formula section of this monograph.

Trade Names

A good deal of this gum is handled and marketed under its common name - guar gum. Trade names for food grade gums include JAGUAR and SuperCol. JAGUAR is the registered trademark of Stein, Hall and Company, Inc. General Mills, Inc. markets SuperCol.

Chemical Abstracts Number

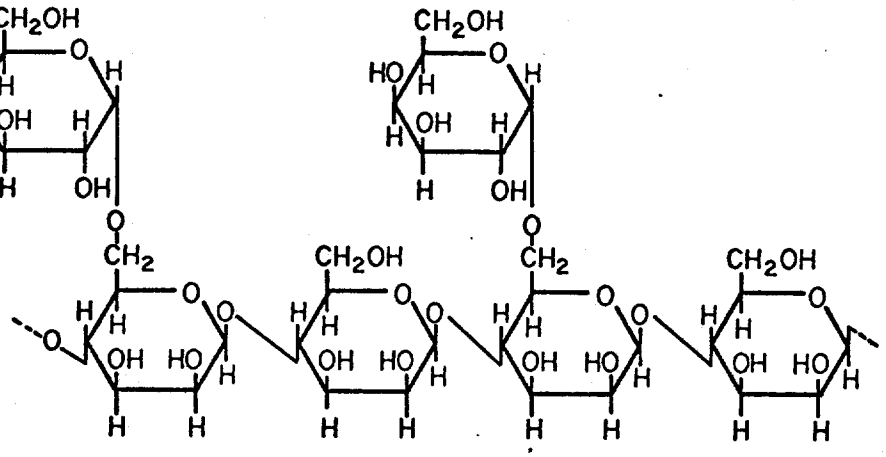
The Chemical Abstracts Services Unique Registry Number is PM9000300.

Empirical Formula

Since guar gum is a polysaccharide its empirical forumla is $(C_6H_{10}O_5)_x$. This formula conveys little specific information, however, since it is representative of the large class of carbohydrate compounds. Guar gum subunits are D-mannose and D-galactose which also have the same empirical formula.

Structural Formula

The structural formula for pure guar gum may be depicted as follows:



This structure is essentially a straight backbone chain of D-mannopyranose units with a side-branching unit of D-galactopyranose on every other D-mannopyranose unit[94,115]. This structural formula is based on physical properties of guar chemical derivatives, stress-strain measurements on gum films, and on X-ray evidence. Some chromatographic evidence indicates that there may be a small percentage of additional branching present in the molecule, but hydrolysis data indicate that the D-mannose to D-galactose ratio is essentially 2:1[94].

The greater degree of branching of guar, as compared to locust bean gum, is believed to be responsible for its easier hydration properties as well as its greater hydrogen-bonding activity[39].

Molecular Weight

The molecular weight of pure guar gum has been reported as around 220,000[70]. An average molecular weight in the range of 200,000 to 300,000 has also been reported[39].

Specifications

Chemical

Chemically pure guar gum would be 100 percent galactomannan. Practically, the best grade of gum would be that prepared from uncontaminated endosperm. A typical analysis of endosperm is as follows:[41]

Protein (N x 6.25)	5 percent	Moisture	10 percent
Ether extract	0.6 percent	Crude fiber	1.5 percent
Ash	0.6 percent		

with galactomannans making up the rest of the composition. Technical grades generally contain more hull and germ contamination. A typical commercial grade analysis range is:[39]

Galactomannan	78-82 percent
Water	10-13
Protein	4-5
Crude fiber	1.5-2.0
Ash	0.5-0.9
Ether extractables (fat)	0.5-0.75
Iron	Trace
Heavy metals	0
Arsenic	0

Food Grade

A typical analysis of impurities in food-grade guar gum is:

Crude fiber	2-5 percent
Moisture	10-15
Protein (N x 6.25)	5-6
Ash	0.5-0.7
Ether extract	0.5-0.8

which indicates it is substantially pure endosperm[41].

Other specifications used by the food industry for guar gum include mesh size of the powder and cold-water viscosity of its solutions.

Food Chemicals Codex Specifications[75]

Galactomannans. Not less than 66.0 percent

Limits of Impurities

Acid-insoluble matter. Not more than 7 percent

Arsenic (as As). Not more than 3 parts per million (0.0003 percent)

Ash (Total). Not more than 1.5 percent

Heavy metals (as Pb). Not more than 20 parts per million (0.002 percent)

Lead. Not more than 10 parts per million (0.001 percent)

Loss on drying. Not more than 15 percent

Protein. Not more than 10 percent

Starch. Passes test, i.e., to a 1 in 10 solution of the gum solution of the gum add a few drops of iodine T.C.: no blue color is produced.

Description

General Characteristics

Guar gum is a free-flowing white to yellowish or grayish white powder, nearly odorless. If an appreciable amount of protein is left in the gum, it can lead to strong odors, spoilage in solution, and enzyme reaction in finished food products[11].

Physical Properties

Guar gum forms a colloidal solution with cold water through rapid hydration to give a highly viscous dispersion; hydration is essentially complete in two hours[41]. Around 90 percent dissolves in water[83]. As an example, a one percent food-grade guar gum solution at 25 C has a viscosity of 3800 centipoises, as measured with a Brookfield No. 3 spindle rotated at 20 r.p.m.[41]. With complete hydration thixotropic properties are obtained at concentrations of one percent or higher. Solutions are less thixotropic below the one percent level, and are only slightly thixotropic at 0.3 percent concentration. Maximum

viscosities are attained at 25 to 40 C. Higher temperatures or prolonged heating cause degradation. Stable and constant viscosities are maintained over a pH range of about 1.0 to 10.5. Hydration is fastest at pH 8 to 9, and slowest above 10 and below 4[39]. The pH of a typical one percent solution is between 5.5 and 6.1 and it tends to become more acid on standing[38].

Aqueous solutions of guar gum are susceptible to biodegradation; the viscosity of an unpreserved solution changes markedly on ageing for one week at around 25 C. Samples containing 0.2 percent benzoic acid maintain reasonably high viscosities over a period of 7 or 8 weeks under similar conditions[78]. The gum is hydrolyzed under strong acid conditions and by enzymic action[49].

The purified gum has an optical activity of $[\alpha]_D^{25}$ + 60° in 0.6 N sodium hydroxide solution[94].

Stability

Guar gum is stable in solid form, and also relatively stable in aqueous solution providing a preservative is used; benzoic acid is recommended[78].

Many polyvalent cations, e.g., Ca^{++}, Al^{+++}, Cr^{+++}, will insolubilize guar gum at certain pH values. It can be protected from this action by sequestering agents[39]. Sodium borate effects rapid gel formation[38].

Analytical Methods

As is true for other natural gums, the isolation and identification of guar gum in food products is a complex problem. Different methods must be worked out for each specific type of food product being examined. Reviews of existing methods are available[33,39,94]. Specific methods for the separation, detection and, in some cases, identification of gums in the following food products have been adopted by the Association of Official Analytical Chemists:[51]

Ice Cream and Frozen Desserts, 16.231-16.237

Mayonnaise and French Dressing, 30.051

Salad Dressing, 30.052-30.054

Soft Curd Cheese, 16.211-16.214

As an example, for frozen desserts the method of isolation involves removal of fat with dioxane, precipitation of protein with tricholoracetic acid, and isolation and purification of the gum by alcohol precipitation. A film of the gum is prepared on a water repellent glass plate, dried and mounted between salt plates, and an infrared spectrum obtained. Different gums can be distinguished by their infrared spectra in the range of about 3 to 15 microns, except that the spectra of guar and locust bean gums are so similar that they cannot be differentiated from one another[51,68]. However, since the principal difference between guar and locust bean gums is that the former has more D-galactose side chains, hydrolysis of the pure sample to obtain the ratio of D-mannose to D-galactose should indicate whether the sample is guar or locust bean gum.

No suitable methods exist for the quantitative determination of guar gum in foods.

Occurrance

Guar gum occurs only in guar plants, more specifically in Cyamopsis tetragonalobus (L) Taub, family Leguminasae. Typically guar gum is around 80 percent of the endosperm of the guar seed. Since the endosperm is about 40 percent of the seed, guar gum is roughly 30 percent of the guar plant seed. The crop has been grown for centuries in India and in what is now Pakistan. It is now grown also in Texas[41].

Guar gum is not produced synthetically.

BIOLOGICAL DATA

Acute Toxicity

Acute toxicity studies for guar gum have not been reported in the literature. Krantz, et al.[61] fed 18 rats guar gum in cocoa butter at a level of 30 percent of their diet for 48 hours with no adverse effects. At 27 percent of diet over a period of 7 days, death in rats (7 of 10) has been reported and attributed to physical blockage of the intestinal tract with no other signs of toxicity apparent at necropsy.[116]

Short-Term Studies

A number of short-term feeding studies have been reported on guar gum in the literature. The general emphasis of these studies has been on the digestibility and caloric value of guar gum rather than toxicity. In only one case was guar gum reported to cause growth depression. This was in chickens at a level of 2 percent of the diet.[62]

Table 1 presents the pertinent data for these studies. Complete synopses are presented in the "Biochemical Aspects" section of this monograph.

Long-Term Studies

No long-term studies on the toxicology of guar gum have been reported in the literature.

Special Studies

No studies have been reported on the mutagenic, teratogenic or carcinogenic effects of guar gum.

TABLE 1. SHORT TERM FEEDING STUDIES

Animal	Sex-No.	Route	Dosage % of diet	Duration	Measurement	Reference
Rat	M-10	oral	27	7 days	wt. gain	116
Rat	M-10	oral	17	7 days	wt. gain	116
Rat	M-5	oral	7.4	7 days	wt. gain digestibility	19
Rat	M-5	oral	6	91 days	wt. gain hematology organ wt.	19
Rat	M-15 F-15	oral	5	6 months	wt. gain histology-liver, kidney	60
Rat	M-15	oral	0.5	21 days	PER	56
Chicken	?-16	oral	3	4 weeks	cholesterol	24
Chicken	?-20	oral	2	3 weeks	wt. gain N, fat absorption Pancreas wt.	62
Monkey	?-2	oral	1g/day	6 months	wt. gain	60

BIOCHEMICAL ASPECTS

Breakdown

No studies have been reported which deal directly with the spontaneous breakdown of guar gum in food under reasonable conditions of storage and processing or cooking. In the dry form it is quite stable. In solution it is susceptible to microbial breakdown unless suitably preserved.[78] The metabolic products of microbial growth have not been reported.

Guar solutions are quite compatible with acids and electrolytes but form heavy curds in the presence of strong alkali.[78]

Absorption - Distribution

The literature on the digestibility and availability of guar gum as used commercially as a food ingredient has been somewhat clouded by the feeding studies reported on guar seed and guar meal. Couch, et al.[22], Couch, et al.[23], and Vohra and Kratzer[109] have reported on the growth inhibiting properties of raw guar meal. These studies are not relevant to the use of guar gum as a foodstuff.

Krantz, et al[61], reported that the addition of 30 percent guar flour to a cocoa butter diet increased the liver glycogen level of fasted rats to 0.8 percent as compared to <0.1 percent in cocoa butter controls. A third group of animals receiving 30 percent wheat flour in cocoa butter had an average liver glycogen level of 2.6 percent.

WARF[116] reported the caloric value of guar gum to be equivalent to corn starch when fed to rats at 17 percent of the diet. At 27 percent of the diet, net weight gain was suppressed. Seven of the ten animals failed to survive the 1 week test period at this level. Other than significantly increased intestinal contents, these animals were judged normal at necropsy.

Booth et al.[19] fed rats a guar gum supplement at a level of 7.4 percent of a maintenance diet and found no significant increase in the weight gain

adjusted for intestinal content. Digestibility of the guar gum was calculated to be 76 percent based on fecal weight increase. Significant variation in net weight gain for control groups reported in this study negates the significance of the results reported for guar gum.

Keane et al.[56] found no significant difference in PER's for rats on a 9 percent protein diet when 0.5 percent guar gum was added at the expense of cellulose in the diet. Significant increases ($P<0.01$) in PER were found when 20 percent water was added to either diet. The addition of 50 percent water also resulted in a significant increase ($P<0.01$) in PER for the guar gum supplemented diet.

Krantz[60] found no significant difference in weight gain between control groups fed a commercial rat ration and those receiving 5 percent guar gum in the diet for a period of 6 months.

Booth et al.[19] found no significant difference in weight gain or food efficiency between rats fed a commercial rat diet and those receiving 6 percent guar gum for a period of 91 days.

Kratzer et al.[62] reported that 2 percent guar gum in the diet of chickens had a deleterious effect on feed consumption, weight gain, nitrogen retention and fat absorption. The results were aggrevated in high protein diets and caused increased pancreas weight.

Krantz[60] also reported on the weight gain of monkeys fed 1g of guar gum per day for 6 months but did not use control animals for comparison.

Synopses for the studies reported above for guar gum are presented below.

Krantz, et al.[61]

Method

Species - rat
Strain - not cited
Sex - male
Age at start of experiment - not cited, 150-200 gm.
Duration of study - 48 hours on experimental diet following a 48 hr fast
Carrier - cocoa butter
Dose schedule - ad libitum feeding

(a) Cocoa butter
(b) Cocoa butter + 30 percent guar flour
(c) Cocoa butter + 30 percent wheat flour

Route of administration - oral
Number of test animals per level -

Cocoa butter controls - 5 groups of 3
30 percent guar flour - 6 groups of 3
30 percent wheat flour - 5 groups of 3

Observations Made

Liver glycogen levels determined at the end of the 48 hr feeding period.

Results

The rats on the cocoa butter diet had less than 0.1% glycogen in the liver while those receiving 30 percent guar flour in the cocoa butter had an average liver glycogen level of 0.8 percent. Liver glycogen levels in the rats with 30 percent wheat flour in the diet were significantly greater with an average of 2.6 percent.

Wisconsin Alumni Research Foundation[116]

Method

Species - rat
Strain - Sprague-Dawley
Sex - male
Age at start of experiment - weanling
Duration of study - 1 week conditioning on basal diet; 1 week experimental
Carrier - Basal diet containing:

Vitamin free casein	42.0 percent
Sucrose	12.5
Cellulose	3.5
Salt mixture	6.5
Starch	29.0
Vitamin mixture	2.5
Cottonseed Oil	4.0
	100.0

Dose schedule - 1g. or 3g. in 5g. basal diet per rat per day (17 and 27 percent of diet).
Route of administration - oral
Number of animals per level - 10

Observations Made

Weight gain - corrected for intestinal weight.
Characteristics of droppings.

Results

Table 2 presents the weight gain data for rats receiving basal diet, basal diet plus corn starch and basal diet plus guar gum. When adjusted for the enlarged large intestine contents of the guar fed animals, the net gain over basal for guar gum was equivalent to corn starch at 17 percent of the diet but was significantly depressed at the 27 percent level.

It is important to note that only 3 of the ten rats fed the 27 percent guar diet survived. No pathology was reported on these animals*. While the animals receiving guar gum appeared bloated and had significantly greater intestinal content, the authors report that all droppings appeared normal with no looseness or binding in any of the animals.

* Personal communication with P. Derse of WARF determined that necropsies were performed and no abnormalities were found. Failure of the animals was attributed to the blockage caused by the excessively large amount of this hydrocolloid ingested.

TABLE 2. WEIGHT GAIN VERSUS DIET

	Average Weight in Grams/10 rats					
	Initial	Final	Total gain	Large intestine contents[a]	Total gain minus intestinal weight	Net gain over basal
1. Basal @ 5gm/rat/day	57.6	57.0	-0.6	1.7	-2.3	--
2. Basal + corn starch @ 1 gm/rat/day	57.5	61.5	4.0	1.7	2.3	4.6
3. Basal + corn starch @ 3 gm/rat/day	57.6	74.4	16.8	2.1	14.7	17.0
4. Basal + guar gum @ 1 gm/rat/day	57.6	64.4	6.8	4.4	2.4	4.7
5. Basal + guar gum @ 3 gm/rat/day	57.6	62.7[b]	5.0[b]	4.8[b]	0.2[b]	2.5[b]

(a) Average for 5 rats only.
(b) For 3 surviving rats.

Booth et al.[19]

Method

Species - albino rat
Strain - not cited
Sex - male
Age at start of experiment - weanling
Duration of study - 7 days
Carrier - basal diet
Dose schedule - 0.4 g/day + 5 g basal diet (7.4 percent of diet)
Route of administration - oral
Number of animals per level - 5

Observations Made

Weight change

(a) 7 days on supplement
(b) 2 days on basal diet following the 7 days on supplement
(c) Net - 9 day weight change for supplemented group minus 9 day weight change for control group on basal diet only.

Fecal weight increase
Digestibility =

$$\frac{\text{total intake of test material - fecal increase}}{\text{Total intake of test material}}$$

Results

The average weight change for the 5 rats receiving the 7.4% guar gum supplement was 6 g as compared to -3 g for the basal diet group. When returned to basal diet for two days the average weight change was -14 g as compared to an additional -6 g for the control group. This resulted in an adjusted net of 1 g for the guar gum supplementation.

Digestibility was calculated to be 76 percent on the basis of fecal increase. As noted by the authors, this is contradictory with the low net weight gain.

In the same paper the authors present data for two other sets of experiments where control groups were on a 5 g maintenance basal diet. The net weight changes for these control groups were +18 g and -2 g as compared to the -9 g for the control group used for the guar gum experiment. This raises serious doubts about the significance of the adjusted net change reported for guar gum.

Keane et al.[56]

Method

Species - rat
Strain - Sprague-Dawley
Sex - male
Age at start of experiment - 21 days
Duration of study - not cited
Carrier - basal diet (9% protein, dwb)

Casein	10.16 g/100 g
Sucrose	71.64
Corn oil	8.00
Vitamin mix	0.10
Choline chloride	0.10
Cellulose	5.0
Mineral mix	5.0
d α-tocopheryl acid succinate	1000 units

Dose schedule - 0.5 percent of diet at the expense of cellulose; ad libitum food and water. Repeated at 0, 20, 50 and 80 percent added water in the diet.
Number of animals per level - 5 per group; 3 replications

Observations Made

Protein Efficiency Ratio (PER)

Results

The addition of water at levels of 20 or 50% to the basal diet containing 0.5 percent guar gum resulted in a highly significant increase ($P < 0.01$) in PER. The same was true for diets supplemented with starch or agar rather than guar. A significant increase ($P < 0.01$) in PER was also found in the absence of any thickener when 20 percent water was added. At 20 percent added water, the PER's were not significantly different between the basal diet group and those receiving 0.5 percent guar gum at the expense of cellulose.

Krantz[60]

Method

Species - rat
Strain - not cited
Sex - male and female
Age at start of experiment - young (?)
Duration of study - 6 months
Carrier - Purina Chow
Dose schedule - 5 percent of diet, ad libitum
Route of administration - oral
Number of animals per level - 15 male, 15 female

Observations Made

Weight gain - weekly weighings
Liver and kidney histology at 2-3 months intervals

Results

No differences were measured in weight gain between the control animals and those receiving 5 percent guar gum. All liver and kidney sections were reported to be normal at 6 months.

Booth et al.[19]

Method

Species - albino rat
Strain - not cited
Sex - male
Age at start of experiment - weanling
Duration of study - 91 days
Carrier - commercially formulated rat diet
Dose schedule - 6 percent of diet, ad libitum
Route of administration - oral
Number of animals per level - 5

Observations Made

Weight gain
food efficiency
hemoglobin
red and white cell counts
organ weights

Results

Mean weight gain for the animals receiving basal diet plus 6 percent guar was not significantly lower than for the animals receiving only the basal ration. Gain/food intake also was not significantly lower than that of the unsupplemented group. No significant alterations were observed in hemoglobin, red cell count or white cell count. Organ weights were normal.

Kratzer, et al.[62]

Method

Species - Chickens
Strain - not cited (Arbor Acres)
Age at start of experiment - 1 day
Sex - not cited
Duration of study - 3 weeks
Carrier - Basal diets (Table 3)
Dose schedule - 2% of diet fed *ad libitum*
Route of administration - oral
Number of test animals per level - duplicate groups of 10

Observations Made

Weight gain
Feed consumption
Nitrogen retention - chromic oxide indicator method[27]
Fat absorption - chromic oxide indicator method
Metabolizable energy - method of Hill and Anderson[50]
Pancreas weight
Bone ash

Results

The effect of guar gum and several other natural and synthetic gums at 2% levels of the conventional basal diet on weight gain, feed consumption, nitrogen retention and fat absorption in chicks and the metabolizable energy of the feed are presented in Table 4. Guar gum had a deleterious effect on all parameters. When repeated with high fat and high protein diets, similar depressing effects were observed with somewhat greater aggrevation in the high protein diet. Guar gum also caused increased pancreas weight in the high protein diet. Lower bone ash and rickets were noted for the growth depressing gums, including guar, when fed in a diet marginally deficient in vitamin D_3.

TABLE 3. COMPOSITION OF THE EXPERIMENTAL DIETS

Ingredient	Low fat basal gm/kg	High fat basal[1] gm	High protein basal gm/kg
Ground milo	270	--	--
Ground yellow corn	300	--	335
Corn starch	--	173	--
Soybean meal (50% protein)	200	288	415
Fish meal (65% protein)	75	105	75
Dried whey	50	50	50
Dehydrated, alfalfa meal (20% protein)	40	40	40
Soybean oil	--	100	20
$CaHPO_4$-$2H_2O$	25	25	25
Cr_2O_3 mix[2]	10	10	10
Sodium chloride[3]	5	5	5
Vitamin mixture[4]	5	5	5
Test material	20	20	20

(1) The total mix was not adjusted to 1 kg.
(2) Mixed in wheat flour dough, baked and ground powder supplied 3gm Cr_2O_3.
(3) Iodized sodium chloride contained 0.025 gm $MnSO_4$-H_2O/5 gm.
(4) Supplies: riboflavin, 1.1 mg; niacin, 1.1 mg; Ca-pantothenate, 1.1 mg; choline chloride, 5.5 mg; folic acid, 33.3 µg; vitamin A, 7,500 I.U.; vitamin D_3, 1,270 I.C.U. (exp. 1 and 3) or 127 I.C.U. (exp. 2); vitamin E, 88 I.U. and bran to make 5 grams.

TABLE 4. EFFECT OF 2% LEVELS OF VARIOUS POLYSACCHARIDES ON WEIGHT GAIN, FEED CONSUMPTION, NITROGEN RETENTION, AND FAT ABSORPTION IN CHICKS, AND THE METABOLIZABLE ENERGY OF THE FEED CONTAINING 1,270 I.C.U./kg VITAMIN D_3

Supplement	Weight gain gm	Feed intake gm	Feed gain	% N retention	% Fat absorbed	Metabolizable energy kcal/gm
Cellulose	299^{d5}	513	1.72^{a5}	51^{bcde5}	76^{cd5}	2.93^{bc5}
Guar gum	149^{a}	358	2.42^{d}	33^{a}	64^{a}	2.44^{a}
Gum carob	180^{ab}	355	1.98^{b}	53^{def}	82^{d}	2.90^{bc}
Gum karaya	229^{bc}	447	1.95^{b}	45^{b}	74^{bcd}	3.08^{c}
Pectin	231^{bc}	410	1.78^{a}	47^{bcd}	79^{cd}	2.89^{bc}
Me-Et-Cel[1]	304^{d}	543	1.79^{a}	58^{f}	79^{cd}	3.13^{c}
Me-Cel[2]	306^{d}	543	1.76^{a}	56^{ef}	76^{cd}	2.92^{bc}
HO-Pr-Cel[3]	275^{cd}	514	1.78^{a}	53^{cdef}	76^{abc}	2.89^{bc}
CM Cel (4 HP)[4]	236^{bc}	466	1.98^{b}	46^{bcd}	65^{a}	2.89^{bc}

(1) Methylethycellulose.
(2) Methylcellulose.
(3) Hydroxypropylcellulose.
(4) Carboxymethycellulose.
(5) Statistical significance at 1% level is denoted by different letters in a column.

Krantz[60]

Method

Species - Macacus Rhesus monkey
Sex - not cited
Age at start of experiment - not cited
Duration of experiment - 28 weeks
Carrier - 1 g in a "finger role"
Dose schedule - 1 g per day
Route of administration - oral
Number of animals per level - 2 (no controls)

Observations Made

Weight gain

Results

Total weight gains of 0.7 and 0.52 kg reported for the two animals. Further results of this study, which was continued, are not available.

Metabolism and Excretion

No studies have been reported on the metabolic fate of guar gum in man or animals.

Effects on Enzymes and Other Biochemical Parameters

Creger, et al.[24] have reported that the addition of 3% guar gum to a basal diet for chicks reduces the cholesterol level of blood serum, liver and spleen. When fed concurrently with 3% added cholesterol, it reduced the increase of serum, liver, spleen, aorta and heart cholesterol. This study is synopsized at the end of this section of the monograph.

Fahrenbach, et al.[34] have also reported hyprocholesterolemic activity for guar gum in the diets of cockerels at levels as low as 0.25 percent. Ershoff and Wells[31] found that 10 percent guar gum in the diet of rats counteracted the increment in liver cholesterol and liver total lipid induced by concurrent feeding of cholesterol. Riccardi and Fahrenbach[88] found that 10 percent guar added concurrently to a casein-sucrose diet with 1% cholesterol and 10% corn oil significantly reduced both serum and liver cholesterol. At 5 percent guar gum the reduction was not significant. At a level of 5 percent, guar did reduce liver cholesterol and liver lipids significantly when the diet was a commercial rat chow with 5 percent added corn oil. Serum cholesterol reduction was not significant in this case.

Kratzer, et al.[62] found that 2 percent guar gum added to a diet marginally deficient in vitamin D_3 led to lower bone ash and rickets in chickens as well as depressed growth. As reported in the "Absorption-Distribution" section of this monograph, this is the only study in the literature where guar gum caused growth depression at this level in the diet. In their study of hypocholesterolemic activity in cockerels, Fahrenbach, et al.[34] reported that 2 percent guar gum in the diet resulted in a slight increase in growth. At 3 percent of the diet a minimal reduction was noted.

Creger, et al.[24]

Method

Species - chicken
Strain - not cited
Age at start of experiment - 1 day
Sex - not cited
Duration of study - 4 weeks
Carrier - Basal diet containing:

Ground yellow corn	- 27.8 percent
Ground milo	- 23.5
Soybean oil meal (44%)	- 40.0
Fish meal (60%)	- 3.0
Dehydrated alfalfa leaf meal (20%)	- 2.5
Di-calcium phosphate	- 2.5
NaCl	- 0.02
Vitamin & mineral premix	- 0.05

Dose schedule - ad libitum

Group 1 - basal diet
Group 2 - basal + 3% guar gum
Group 3 - basal + 3% cholesterol
Group 4 - basal + 3% guar gum + 3% cholesterol

Route of administration - oral
Number of test animals per level - duplicate groups of eight

Observations Made

Cholesterol level at four weeks of:

blood serum
liver
spleen
aorta - thoracic and abdominal
heart
brain

Results

Table 5 presents the results for the different treatment groups. The inclusion of 3 percent guar gum in the basal diet significantly reduced the blood serum cholesterol level, liver cholesterol and spleen cholesterol. The increase in serum, liver and spleen cholesterol induced by incorporation of 3 percent cholesterol in the diet was also significantly reduced by concurrent supplementation of 3 percent guar gum.

In the aorta and the heart the addition of guar gum to the basal diet did not have a significant effect on cholesterol level. However, 3 percent guar gum did significantly reduce the increase of cholesterol level caused by the addition of 3 percent cholesterol when fed concurrently. Guar gum had no effect in lowering brain cholesterol in either the basal or atherogenic diet.

TABLE 5. THE EFFECT OF GUAR GUM ON CHOLESTEROL LEVELS OF VARIOUS BIOLOGICAL SAMPLES OF CHICKS FOUR WEEKS OF AGE

	Treatment Groups			
	1	2	3	4
	Supplements to the Basal Diet			
Sample	None	3% Guar Gum	3% Cholesterol	3% Guar Gum + 3% Cholesterol
	Cholesterol	Cholesterol	Cholesterol	Cholesterol
	mg%	mg%	mg%	mg%
Blood Serum	99.7^{b}	77.1^{a}	180.7^{c}	112.4^{b}
Liver	369.9^{b}	326.0^{a}	549.0^{d}	432.6^{c}
Spleen	395.6^{b}	335.9^{a}	494.1^{d}	424.5^{c}
Aorta	195.6^{b}	176.6^{ab}	224.1^{c}	166.7^{a}
Heart	207.1^{a}	224.8^{a}	262.8^{b}	223.5^{a}
Brain	$1,892.2^{a}$	$1,897.1^{a}$	$1,939.6^{a}$	$1,905.6^{a}$

Values with different subscript letters differ significanlty at the 0.05% level as determined using Duncan's Multiple Range Test (Duncan, 1955).

Interaction with Food and Drugs

Other than the effects on serum and organ cholesterol levels as reported in the previous section of this monograph, no data exists on the possible interaction of guar gum with food materials or drugs. Guar gum is of course a very effective hydrocolloid and does bind large amounts of water *in vitro*. The significance of this water binding property *in vivo* has not been studied. Unless the gum is fed dry in substantial amounts, it would not be expected to have a significant effect.

CONSUMER EXPOSURE INFORMATION

While originally introduced as a substitute for locust bean gum, guar gum now is widely used in many food products. In 1970, a total of 4,313,388 pounds was reported as used in food products by 39 companies responding to the survey.[10] A detailed survey of food uses and levels of usage is now in progress by NAS/NRC. When completed, these data will allow estimates to be made of the potential daily exposure of consumers to guar gum.

BIBLIOGRAPHY

(1) Agrawal, K.M.L., and Om P. Bahl. 1968. Glycosidases of Phaseolus vulgaris. II. Isolation and general properties. J. Biol. Chem., 243 (1):103-111.

(2) Ahmed, Z.F., and K.M. Nagib. 1962. Guar flour utilization as a stabilizer in ice-cream making and its effect on some physical and microbiological properties of the product. Egypt Pharm Bull 44 (4): 11-18.

(3) Ali, S.M., M. Hanif, and A.S. Ali. 1964. A protein food based on a blend of gram flour, guar seed (Cyamopis tetragonoloba) flour and fish flour. Pakistan Journal of Science and Research 16(3/4):103-104.

(4) Alvi, A.S., M. Hanif, and S.M. Ali. 1964. Net protein utilization of guar (Cyamopis tetragonoloba) treated in different ways and enhancement of its protein value by supplementation. Pakistan Journal of Science and Research 16(1):1-4.

(5) Ambegaokar, S.D., J.K. Kamath, and V.P. Shinde. 1969. Nutritional studies in Protein of 'Gawar' (Cyamopsis tetragonoloba). Journal of Nutrition and Dietetics 6(4):323-328.

(6) Anonymous. 1962. Cream cheese: identity, label statement of optional ingredients. Fed. Regist. 27:3254.

(7) Anonymous. 1965. Cold-pack cheese food: order amending standard by listing guar gum as an optional ingredient. Fed. Regist. 30:3526-3528.

(8) Anonymous. 1966. Identity standards; carrageenan as optional ingredient in cream cheese, and guar gum and carrageenan as optional ingredients in Neufchatel cheese. Fed. Regist. 31:14349-14350.

(9) Anonymous. 1969. Food additives. Components of paper and paperboard in contact with aqueous and fatty foods. Fed. Regist. 34(156):13273-13274.

*(10) Anonymous. 1972. Annual poundage reported per substance by NAS & FEMA user firms. Unpublished preliminary report, Appendix A Part I, Table 1, p. 8. NAS, Washington, D. C.

*(11) Anonymous. Water soluble gums. Tragacanth Importing Corporation, New York, 28 p.

(12) Bakshi, Y.K. 1967. Studies of toxicity and processing of guar meal. Diss. Abstr. 27(7):2268.

(13) Baldwin, R.E., R. Upchurch and O.J. Cotterill. 1968. Ingredient effects of meringues cooked by microwaves and by baking. Food Technology 22: 77-80.

(14) Baur, F.J. 1965. Dry mix for frozen desserts. The Proctor and Gamble Co. (Cincinnati, Ohio) U.S. Patent 3,183,098.

(15) Bayfield, E.G. 1958. Gums and some hydrophilic colloids as bread additives. Baker's Digest 32(3):42-45,73.

(16) Berge, V., and E. Hjersing. 1969. Surface treatment of foodstuffs and food packaging materials. Norwegian Patent Application 116 883.

(17) Blethen, J. 1969. Synthetic pulp for fruit juices. U. S. Patent 3,446,625.

(18) Boissier, J. R. and F. Mendy. 1966. Dietetic Compositions. Societe Industrielle pour la Fabrication des Antibiotiques (S.I.F.A.). Fr. M3789.

*(19) Booth, A. N., A.P. Hendrickson, and F. DeEds. 1963. Physiologic effects of three microbial polysaccharides on rats. Toxicology and Appl. Pharmacol. 5:478-484.

(20) Casares, R. and C. Lopez Herrera. 1960. Bromatological study of Cyamopsis psoraloides, Stizolobium deeringianum, and Phaseolus radiatus, new legumes acclimated to Spain. Anales. bromatol. (Madrid). 12, 405-410.

(21) Chandriami, M. L. 1957. Guar gum. Review of the properties and uses of guar gum (Cyamopsis tetragonolobus). Paintindia 7, No. 9, 34.

*(22) Couch, J.R., Y.K. Bakshi, T.M. Ferguson, E.B. Smith, and C. R. Creger. 1967. The effect of processing on the nutritional value of guar meal for broiler chicks. Brit. Poultry Sci. 8(4):243-250.

*(23) Couch, J.R., C.R. Creger, and Y.K. Bakshi. 1965. Trypsin inhibitor in guar meal. Proc. Soc. Exptl. Biol. Med. 123(1), 263-265.

*(24) Creger, C.R., V.A. DeGuzman, and J.R. Couch. 1970. The effect of guar gum on cholesterol levels of chicks. Nutr. Report Internat'l., 2(5):243-247.

(25) Czaja, A. Th. 1962. Microscopic detection of some commonly used thickeners in dry mixes. Z. Lebensm.-Untersuch.-Forsch. 117, 499-513.

(26) Dange, S.R.S. 1969. Effect of relative humidity and period of storage on germination of Guar (Cyamopsis tetragonoloba Taub.) seeds. Bulletin of Grain Technology 7(2):121-122.

*(27) Dansky, L.M., and F.W. Hill. 1952. Application of chromic oxide indicator method to balance studies with growing chickens. J. Nutr. 47:449-459.

(28) Dijkstra, N.D. 1962. Food value of pre-treated guar meal. Versl. landbouwkund. Onderz. Nr. 68.11, 822-830.

(29) Doane, W.M. and R.L. Whistler. 1963. Comparison of the anticoagulant activity of three polysaccharide sulfates. Arch. Biochem. Biophys. 101, 436-438.

(30) Elenbogen. G.D. 1968. Edible dietary spread. U.S. Patent 3,397,995.

*(31) Ershoff, B.H., and A.F. Wells. 1962. Effects of gum guar, locust bean gum, and carrageenan on liver cholesterol of cholesterol-fed rats. Proc. Soc. Exptl. Biol. Med., 110:580-582.

(32) Esser, J. 1957. Guar - a versatile crop. Seed World 80(6):12, 15.

*(33) Ewart, M.H., and R.A. Chapman. 1952. Identification of stabilizing agents. Anal. Chem. 24:1460-1464.

*(34) Fahrenbach, M.J., B.A. Riccardi, and W.C. Grant. 1966. Hypocholesterolemic activity of mucilaginous polysaccharides in white leghorn cockerels. Proc. Soc. Exp. Biol. and Med., 123:321-326.

(35) Fouassin, A. 1957. Alginates, carboxymethylcelluloses, and other thickening agents in derivatives of milk and mayonnaises. Rev. fermentations et inds. aliment 12:169-172.

(36) Not used.

(37) Garg, S.S.P., S.R.T. Thampy, and N.R. Kuloor. 1961. Extraction of Vegetable Gum. Ind. P. 69 344.

*(38) Glicksman, M. 1966. Gums, natural. p. 750. In Kirk-Othmer, Encyclopedia of Chemical Technology, 2nd Ed., Vol. 10, Wiley, New York.

*(39) Glicksman, M. 1969. Gum technology in the food industry. Academic Press, New York, 590 pp.

(40) Glicksman, M. 1962. Using natural polysaccharide gums in the food industry. Advances Food Res., 11:109-200.

*(41) Goldstein, A.M., and E.N. Alter. 1959. Guar gum, p. 321-341, In Whistler, R.L., and J.N. BeMiller, Industrial gums. Academic Press, New York.

(42) Gouveia, A.P., and A.J.A. de Gouveia. 1962. Dried fish of Angola. Some vitamins and the amino acid composition of the proteins. Estudos Cient. Homenagem J. Carrington da Costa, Lisbon, 41-65.

(43) Grindrod, J. and T.A. Nickerson. 1968. Effect of various gums on skimmilk and purified milk proteins. J. Dairy Science 51 (6), 834-841.

(44) Haksar, C.N., and S.G. Kendurkar. 1959. Analysis and uses of guar gum. Vikram, 3(1):80-83.

(45) Hanif, M., A.S. Alvi, and S.M. Ali. 1964. A protein food (for man) based on a blend of gram flour, guar seed flour and skimmed milk powder. Pakistan Journal of Science and Research, 16(1):37-38.

(46) Hanif, M., M.Awais, and S.M. Ali. 1965. Supplementary value of a protein food (PF 1) based on guar, gram and fish flours to certain indigenous cereals. Pakistan Journal of Science and Research, 17(3/4): 132-134.

(47) Haug, A., and O. Smidsroed. 1968. Solubility of polysaccharides in salt solutions. Chem. Soc., (London), Spec. Publ., No. 23, 273-282.

(48) Henderson, N.L., and L.N. Elowe. 1965. Sustained release encapsulated formula with a hydrophilic gum. U.S. Patent 3,427,378.

*(49) Heyne, E., and R.L. Whistler. 1948. Chemical composition and properties of guar polysaccharides. Jour. Amer. Chem. Soc., 70:2249.

*(50) Hill, F.W., and D.L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. J. Nutr. 64:587-603.

*(51) Horwitz, W., ed. 1970. Official methods of analysis of the Association of Official Analytical Chemists. 11th Ed., Association of Official Analytical Chemists, Washington, D.C. 1015 p.

(52) Jackson, G.J., Jr., and I.S. Butensky. 1967. Dispersible guar gum. U. S. Patent 3,313,800.

(53) Johnson, R.H. 1956. Determination of gums in process cheese spreads. J. Assoc. Offic. Agr. Chemists, 39, 286-290.

(54) Kawatra, B.L., J.S. Garcha, D.S. Wagle. 1969. Studies on guar (Cyamopsis tetragonoloba) protein. J. Nutr. Diet. 6(2):91-97.

(55) Kawatra, B.L. and G.A. Sidhu. 1968. Studies on the effect of feeding guar meal to rats. J. Nutr. Diet. 5(1):57-60.

*(56) Keane, K.W., C.J. Smutko, C.H. Krieger and A.E. Denton. 1962. The addition of water to purified diets and its effect upon growth and protein efficiency ratio in the rat. J. Nutr. 77:18-22.

(57) Keen, J.L. 1969. Low-odor, low-taste galactomannan gums. U. S. Patent 3,455,899.

(58) Keller, H.M. 1968. Mixture for the preparation of fried potato. Fr. 1,539,678.

(59) Klostermann, R.H. 1963. Stabilizing composition for oil-in-water emulsions. U.S. Patent 3,108.004.

*(60) Krantz, J.C., Jr. 1946. Feeding of guar flour to rats (life span) and monkeys. Preliminary report to General Mills, Inc. 6 p.

*(61) Krantz, J.C., Jr., C.J. Carr and deC.B. Farson. 1948. Guar polysaccharide as a precursor of glycogen. J. Amer. Diet. Assoc. 24:212.

*(62) Kratzer, F.H., R.W.A.S.B. Rajaguru, and P. Vohra. 1967. The effect of polysaccharides on energy utilization, nitrogen retention and fat absorption in chickens. Poultry Science, 46(6):1489-1493.

(63) Lenderink en Co. N.V. 1969. Method for producing a whipped product containing an edible fat. Netherlands Patent Application 6 907 938.

(64) Light, J.M., G.K. Stookey, and J.C. Muhler. 1970. The effects of sodium alginate and other untested polymers on radiostrontium retention in the rat. Proc. Soc. Exp. Biol. and Med., 133(4):1259-1264.

(65) Little, L.L. 1968. Imitation sour cream. U.S. Patent 3,391,002.

(66) Malik, M.Y., A.A. Sheikh, S. Akhtar, and I. Ahmad. 1967. Digestibility of guar meal in sheep. Agr. Pakistan 18(3):333-339.

(67) Matlock, R.S. 1960. Guar variety and cultural studies in Oklahoma. 1950-1959. Oklahoma Agric. Expt. Sta. Processed Ser./P-366, 1-37.

*(68) McNulty, J.A. 1960. Isolation and detection of gums in frozen desserts. J. Assoc. Offic. Agr. Chemists., 43, 624-632.

(69) McNulty, J.A. 1961. Collaborative study of gum in ice cream mix. J. Assoc. Offic. Agr. Chemists, 44, 513-516.

*(70) Meer, G., Jr., and W.A. Meer. 1962. Natural plant hydrocolloids. Am. Perfumer, 77, II. (4):49-52.

(71) Mehta, D.R., and C.V. Ramakrishnan. 1957. Studies on guar seed oil (Cyamopsis psoralloides). Jour. Amer. Oil Chemists' Soc. 34(9):459-461.

(72) Menzi, R.F. 1963. Edible low caloric composition and process of production. U. S. Patent 3,097,946.

(73) Mital, S.P., V. Swarup, M.M. Kohli, and H.B. Singh. 1969. Variability in guar. Indian J. Genet Plant Breed, 29(1):98-103.

(74) Namba, T., I. Tada, and S. Takahashi. 1967. Pharmacognostical studies on the water soluble seed gums. Syoyakugaku Zasshi 21(1):47-56.

*(75) National Academy of Sciences - National Research Council, Food Protection Committee, Committee on Specifications of the Food Chemical Codex. Revised proof for the 1972 edition, Washington, D.C.

(76) Oke, O. L. 1964. Hydrocyanic acid content and nitrogen-fixing capacity of guar (Cyamopsis tetragonoloba). Nature, 204(4956):405-406.

(77) Organon, N.V. 1955. Pharmaceutical preparations for gastric hyperacidity. Dutch 77,072.

*(78) Osborne, G.E. and C.O. Lee. 1951. A study of guar and its mucilages, I. Pharmaceutical preparations. II. The effects of certain factors on the viscosity of guar and other mucilages. Bull. Nat'l. Formulary Committee, 19 (No. 1-2):4-19.

(79) Pader, M., H.D. Hamilton, J.J. Miles, Jr., and G.J. McCrimlisk. 1956 Production of Meat-type Sandwich Spread. A.P. 2 874 049.

(80) Patel, B.M., B. M. Dhami, and P.C. Shulka. 1970. Nutritive value of guar seed (crushed) and guar meal. Indian Vet. J. 47(1):66-70.

(81) Patton, T.C. 1969. Viscosity profile of typical polysaccharides in the ultra-low shear rate range. Cereal Science Today 14 (5):178-183.

(82) Proszynski, A.T., A. J. Mitchell, and C.M. Stewart. 1965. Australian plant gums. I. Classification and identification of gums from arborescent angiosperms. Australia, Commonwealth Sci. Ind. Res. Organ., Div. Forest Prod. Technol. Paper, No. 38, 19 pp.

*(83) Rafique, C.M., and F. Smith. 1950. The constitution of guar gum. J. Amer. Chem. Soc., 72:4634-4637.

(84) Rahman, M.S., and R.E. Leighton. 1968. Guar meal in dairy rations. J. Dairy Sci., 51(10):1667-1671.

(85) Ramakrishnan, C.V. 1957. Amino acid composition of crude and gerimated guarseed flour protein (Cyamopsis psoralloides). Experientia 13(2): 78-79.

(86) Reinders, M.A., Gotlieb, K.F. 1969. Mayonnaise-like dressings and sauces. Neth. Appl. 67 15,194.

(87) Rheinlaender, A.P. 1968. Improvement of the taste of artificially sweetened foods with glutamates. Ger. 1,266,623.

*(88) Riccardi, B.A., and M.J. Fahrenbach. 1967. Effect of guar gum and pectin N.F. on serum and liver lipids of cholesterol-fed rats. Proc. Soc. Exp. Biol. Med. 124 (3):749-752.

(89) Roaf, A.L., and P.M. Brickley, Jr. 1969. Method for the extraction of light filth from plant gums. J. Assoc. Offic. Anal Chem. 52(1): 17-18.

(90) Saxena. V.K. 1965. Guar gum, a versatile product. Res. Ind (New Delhi) 10(4):101-106.

(91) Schlegel, R.A., C.M. Gerbeck, and R. Montgomery. 1968. Substrate specificity of D-galactose oxidase. Carbohyd. Res. 7(2), 193-199.

(92) Shah, S.S.I., M.B. Sial, and B.H. Schneider. 1966. The digestibility of guar meal. Agr. Pakistan 17(1):35-39.

(93) Singer, R.L. 1969. Synthetic food products. West German Patent Application 1 903 644.

*(94) Smith, F., and R. Montgomery. 1959. The chemistry of plant gums and mucilages. Reinhold Publishing Corporation, New York. ACS Monograph Series No. 141, 627 p.

(95) Sobrie, J. 1963. Analysis of Fruit-juices (Oranges, Lemons and Grapefruits). Fermentatio (Gand) 25, 211-18.

(96) Spitzer, J.G., L.S. Nasarevich, J.L. Lange, and H.S. Bondi. 1957. Mayonnaise-type foodstuffs. A.P. 2 944 906.

(97) Srivastava, S.R., and G.S. Singh. 1960. Digestibility coefficient and nutritive value of guar meal or churi. Indian Jour. Dairy Sci. 13(3): 136-142.

(98) Strange, T.E. 1957. Detection of gums in catsup and related tomato products. J. Assoc. Offic. Agr.Chemists 40, 482-486.

(99) Subramaniam, A. and S. Premsekhar. 1960. Seed production in guar (cluster beans) Cyamopris psoraloides. Madras Agric. Jour. 47(3): 134-135.

(100) Swanson, J.W. 1949. Methylation of guar polysaccharide. Jour. Amer. Chem. Soc., 71(4):1510-1511.

(101) Szczesniak, A.S., and E. Farkas. 1962. Objective characterization of the gustatory behavior of solutions of thickening agents. J. Food Sci., 27, 381-385.

(102) Tatter, C.W., P.P. Noznick. 1969. Acid flavoured vegetable powders. Canadian Patent 823 607.

(103) Tracey, M.V. 1966. Basis of improver action of flour doughs. Chem. Ind. (London) (2), 68-69.

(104) Ud-Din-Ala, M.B. Sial, and B.H. Schneider. 1965. Fattening of cattle. The effect of substituting guar meal for cottonseed cake as a protein supplement. Agr. Pakistan. 16(1):67-74.

(105) Upchurch, R. and R.E. Baldwin. 1968. Guar gum and triacetin in meringues and a meringue product cooked by microwaves. Food Technology 22 (10), 107-108.

(106) Vaisey, M., R. Brunon, and J. Cooper. 1969. Some sensory effects of hydrocolloid sols on sweetness. J. Food Sci., 34(5):397-400.

(107) Vohra, P. and F.H. Kratzer. 1964. The use of guar meal in chicken rations. Poultry Sci., 43(2):502-503.

(108) Vohra, P. and F.H. Kratzer. 1964. Growth inhibitory effect of certain polysaccharides for chickens. Poultry Sci., 43(5):1164-1170.

*(109) Vohra, P. and F.H. Kratzer. 1965. Improvement of guar meal by enzymes. Poultry Sci., 44(5):1201-1205.

(110) Weinstein, B. 1958. Stabilizers for ice cream-type desserts. U.S. Patent 2,856,289.

(111) Werbin, S.J. 1950. Ice cream stabilizer. U.S. Patent 2,502,397.

(112) Werbin, S.J. 1953. Vegetable gums, their properties and uses. Baker's Digest, 27(4):71-73.

* (113) Whistler, R.L. 1954. Guar gum, locust bean gum, and others, p 45-49. In. Natural plant hydrocolloids, Advances in Chem. Series. 11, Amer. Chem. Soc.

(114) Whistler, R. L., and J. W. Marx. 1965. Guaran. Isolation of galaciomannan from guar (Cyamopsis tetragonoloba) seed endosperm. Method. Carbohyd. Chem. 5:143.

* (115) Whistler, R.L. and C. L. Smart. 1953. Polysaccharide chemistry. Academic Press, New York,pp. 1-493.

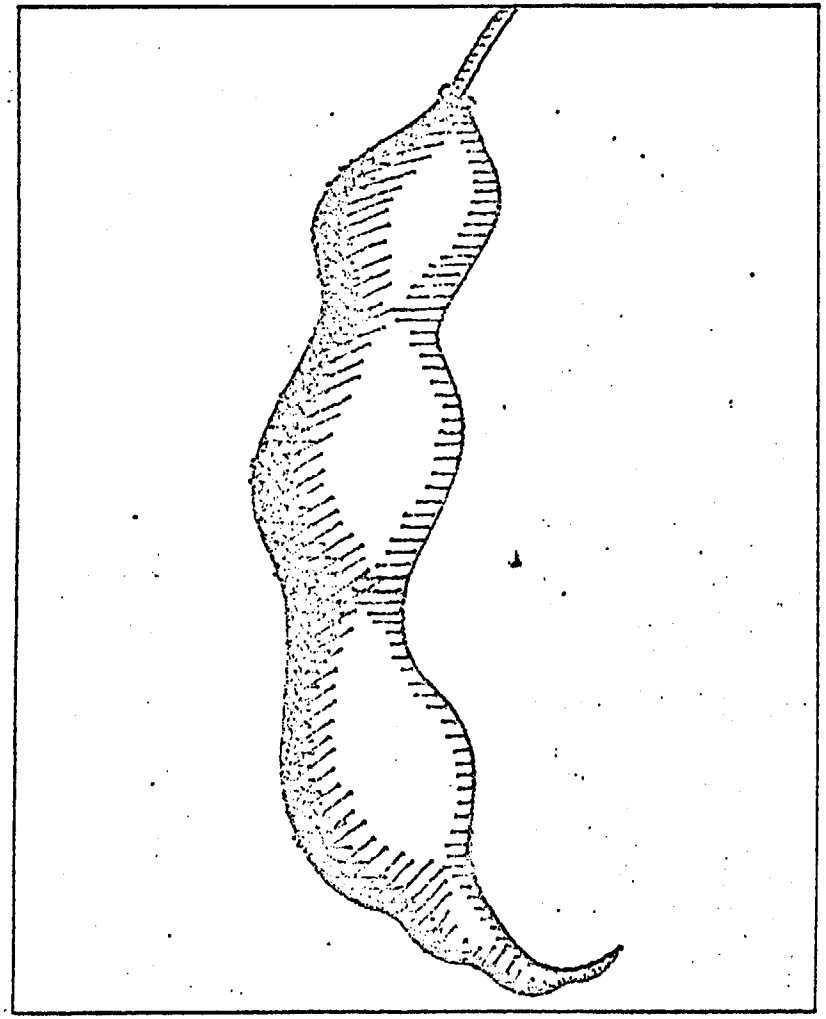
* (116) Wisconsin Alumni Research Foundation. 1964. Assay Report Nos. 3110860 and 3110861.

(11)

TIC
The Integrity Counts

Water Soluble Gums

TIC Pretested Seed Gums



T'I'C Pretested Gum Guar

Gum Guar is derived from the endosperm of a seed found in the pods of a plant known as Cyamopsis tetragonalobus. The plant is cultivated commercially in India and Pakistan.

In order to process the gum, the seeds are separated from the pod and then the seed coating is removed during a series of heating and milling operations. The contents of the seed consist of the endosperm (carbohydrate —Gum Guar) and the germ (rich in protein and enzymes). The endosperm is mechanically separated from the germ by a milling process and is then further processed into various grades of Gum Guar.

If a high percentage of protein (the germ) is left in the Gum Guar, it can lead to development of strong odors, spoilage in solution, and enzyme reaction in finished food products. The exclusive T'I'C process ensures that a minimum amount of protein is left in the finished Gum Guar.

Gum Guar hydrates rather rapidly in cold water and can attain maximum viscosity without the aid of heat or agitation. T'I'C prepares a number of different grades of Gum Guar in various mesh sizes which attain hydration over a wide range of rates.

T'I'C food grades of Gum Guar develop viscosities from 3,200 to 6,500 centipoises at a concentration of 1 percent in water. The pH of a one percent solution of Gum Guar is in the range of 5.5 to 6.1.

Solutions of Gum Guar exhibit a slight buffering action and are relatively stable over a wide pH range (4 to 10). Gum Guar is sensitive to salts in solutions since they affect the hydration rate. Alkaline solutions or borates have a particularly strong effect and form rubbery gels with Guar at relatively low concentrations.

The structure of Gum Guar has been elucidated and it is composed of a straight chain polymer of D-mannose units and D-galactose chains. The molecular weight is approximately 220,000.

Gum Guar is used in a number of varied applications due to its unique hydration and high viscosity properties. Some of these uses are outlined as follows:

Pharmaceutical and Cosmetic

Binder and disintegrant in tablets and pills.

In appetite depressants—the gum swells in the stomach fluids to form a bulky gel.

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THE EFFECT OF PROCESSING ON THE NUTRITIONAL VALUE OF GUAR MEAL FOR BROILER CHICKS

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SYNOPSIS

Guar meal contains two factors which are detrimental to the growth rate and food conversion of chicks. One of the factors is the trypsin inhibitor which can be destroyed by cooking the raw guar meal for a period of 1 hr at a temperature of 110° C. with the injection of super-heated steam for a period of 15 min. after the cooker attains a temperature of 110° C. The second deterimental factor in guar meal is the guar gum which causes a depression in growth and sticky droppings when the total level of the gum in the diet exceeds 1·8 per cent. The usage of heated guar meal in chick diets is apparently limited by the gum content of the meal. Pectinase, an enzyme preparation added to a chick ration which contained 15 per cent processed guar meal, produced an increase in the weight gain of chicks fed the ration over those fed 15 per cent processed guar meal. Hypertrophy of the pancreas resulted in the chicks fed 20 and 30 per cent processed guar meal and in all groups fed raw guar meal.

INTRODUCTION

Cyamopsis tetragonolobus (guar) is a drought resistant annual legume. The endosperm of the guar seed is a rich source of a galactomannan polysaccharide (guar gum). The residue (guar meal) remaining after extraction of the gum contains 35-45 per cent protein and offers a possible source of amino acids in food.

Guar meal was found to have a detrimental effect on chick growth in this laboratory in 1951 (Couch and Dieckert, unpublished data). Vohra and Kratzer (1964*a*, *b*) reported that the substitution of 7·5 per cent guar meal in chick diets caused a depression of growth, a major portion of which was attributed to the presence of the remaining polysaccharide in the meal. Chick growth was reduced 25-20 per cent by the inclusion of 2 per cent guar gum in the chick diet. Anderson and Warnick (1964) reported that the substitution of 10 per cent guar meal in chick diets had an unfavourable effect on growth rate and efficiency of food conversion and that the droppings of the chicks were sticky. Vogt and Penner (1963) substituted 5, 10 and 15 per cent guar meal in broiler diets and reported that growth and food conversion were influenced unfavourably, particularly at the 10 and 15 per cent levels. Van Etten, Miller and Wolff (1961) stated that the amino acid pattern of guar meal was such that this product should provide a good source of amino acids for use in diets for chicks and laying hens. The processing history of the guar meal used in the nutrition experiments reviewed above cannot be determined from the data in the papers.

Osborne and Mendel (1917) reported that the nutritional value of the soyabean was improved by autoclaving. Since that time the nutritional value of a number of legume seeds has been reported to be improved by autoclaving (Orru and Damel, 1941; Sure and Read, 1921; Everson and Hackert, 1948; Richardson, 1948). It is a well recognised fact that the heating of soyabean meal improves the nutritional value of the meal (Wilgus, Morris and Hueser, 1936; Hayward, Halpin, Holmes, Bohstedt and Hart, 1937). A crystalline substance which inhibited the proteolytic activity of trypsin has been isolated from defatted soyabeans (Kunitz, 1945, 1946). Guar meal was not improved by autoclaving according to the report of Borchers and Ackerson (1950).

Experiments to be reported herein were designed to study the effect of heat on the nutritional value of raw guar meal and on the amino acid pattern of the meal.

MATERIALS AND METHODS

Processing of guar meal. Raw guar meal[1] was processed initially in a small laboratory cooker in the quantity of 600-700 g. after the method of Lyman, Holland and Hale (1944). The design of the cooker permitted the injection of superheated steam at any time during the processing of the meal. The heat-treatment of the meal used in nutritional studies is described in the various experiments which follow.

Broiler chick studies. Broiler cross chicks were used in all tests reported. The chicks were distributed at random among the various experimental groups at one day of age and were maintained in electrically heated batteries with raised screen floors for the experimental period indicated. When the duration of the tests exceeded 4 weeks, the birds were moved to growing batteries with raised screen floors and without heat. Records of food consumption and mortality were kept. Food and water were supplied *ad libitum*. The basal diet had the following percentage composition: ground yellow corn, 27·5; milo, 21·1; soyabean meal (45 per cent protein), 39·2; dehydrated alfalfa meal (20 per cent protein), 2·5; Menhaden fish meal (60 per cent protein), 3·0; defluorinated rock phosphate (19 per cent phosphorus and 32 per cent calcium), 2·5; salt, 0·2; vegetable oil, 3·0; and vitamin-trace mineral premix, 1·0. The vitamin-trace mineral premix supplied the following per kg. of diet: vitamin A, 6600 i.u.; vitamin D_3, 2200 i.c.u.; vitamin E, 5·5 i.u.; riboflavin, 4·4 mg.; D-calcium pantothenate, 11 mg.; niacin, 33 mg.; chlortetracylcine, 22 mg.; vitamin B_{12}, 12 μg.; choline chloride, 660 mg.; manganese, 55 mg. and zinc, 55 mg. The guar meal used in these studies contained 42·5 per cent protein by analysis. Diets were maintained on an isonitrogenous basis when guar meal was substituted into the basal diet by adjusting soyabean oil meal and milo levels.

Experiment 1. Day-old male chicks were distributed at random into two replicates of 16 chicks each in experiments 1 and 2. The birds were fed raw guar meal, meal that had been heated at 110° C. for 30 min., and that which had been heated at 110° C. for 30 min. with super-heated steam injected at 110° C. for a period of 15 min. The guar meal was substituted for soyabean meal at levels of 10 and 20 per cent on an isonitrogenous basis. One treatment consisted of feeding the chickens the basal diet unsubstituted as a positive control. Experiments 1 and 2 were terminated at 4 weeks of age.

Experiment 2. Guar meal which had been processed in the laboratory by heating for ½, 1, 1½ or 2 hr at a temperature of 110° C. with the injection of super-heated steam at 110° C. for 15 min. was substituted for soyabean meal on an isonitrogenous basis in the basal diet at a level of 10 per cent.

[1] All guar meals used in the study were supplied through the courtesy of Pakistan Gum and Chemicals, National and Grindlays Bank, McLeod Road, Karachi 2, Pakistan.

Experiment 3. The third experiment was set up primarily to determine the effect of 10, 20 and 30 per cent raw guar meal on pancreas hypertrophy. There were seven groups of 20 chicks each (mixed sexes) used in this study. The experiment was terminated when the chicks were 25 days of age at which time all chicks were weighed, killed, and the pancreas removed and weighed after blotting with a paper towel.

Experiment 4. Day-old female chicks were randomly distributed into two replicates of 16 chicks each. Commercially processed guar meal, which had been treated by heating at 110° C. for 1 hr with the injection of super-heated steam for 15 min. at 110° C., was substituted for soyabean meal in the basal diet at levels of 2½, 5, 7½, 10, 12½ and 15 per cent. At the end of 4 weeks the birds were weighed and transferred from starter batteries to grower batteries. The experiment was terminated when the birds were 8 weeks of age.

Experiment 5. Eight male and 8 female broiler cross chicks were distributed at random into four replicates. Commercially processed guar meal, which had been treated by heating at 110° C. for a period of 1 hr with the injection of super-heated steam at 110° C., was substituted for soyabean meal in the basal diet at levels of 10 and 15 per cent. Guar gum and guar splits, the raw material from which guar gum is made, were each substituted into the basal diet for milo at levels of 1·8 and 2·7 per cent respectively. The duration of the experiment was 4 weeks.

Experiment 6. In this experiment 15 per cent commercially processed guar meal, prepared as described in experiment 5, was substituted for soyabean meal in the chick ration, and 0·2 per cent pectinase was added in addition to the 15 per cent processed guar meal to determine if pectinase would improve the digestibility of the guar gum residue in the guar meal. There were 13 chicks per group, and each treatment was replicated twice for the 4-week period.

EXPERIMENTAL RESULTS

Experiment 1. The substitution of 10 or 20 per cent raw guar meal for soyabean meal reduced growth significantly ($P<0.01$) in broiler cross chicks (Table 1). Growth of the chicks was improved when the raw guar meal was heated for a period of 110° C. for 30 min. A further improvement was noted when the meal was processed with the injection of super-heated steam for 15 min.

TABLE 1

Experiment 1: The effect of substituting 10 or 20 per cent raw or processed guar meal for soyabean meal on the average weight of broiler cross chicks at 4 weeks of age

Treatment	10 per cent meal Average wt at 4 weeks (g.)	20 per cent meal Average wt at 4 weeks (g.)
Raw guar meal	401 (1)[1]	186 (4)
Processed at 110° C. for 30 min.	430 (2)	302 (5)
Processed at 110° C. for 30 min. and injected steam at 110° C. for 15 min.	453 (3)	330 (6)
Control with soyabean meal	495 (7)	

[1] Group number indicated in parentheses.
Statistical comparison of means (Duncan, 1955)
Chick weights at 4 weeks $P<0.05$ 7 3 2 1 6 5 4.

A significant decrease in the 4 week weights of broiler cross chicks resulted by increasing the guar meal in the diet from 10 to 20 per cent of the ration. The beneficial effect of heating the meal on the growth of the chicks was much more apparent when the level of usage was 20 per cent (Table 1).

Experiment 2. From the results of experiment 1, an indication was obtained to the effect that best growth results could be obtained by processing the guar meal 110° C. for a period of 1 hr with the injection of super-heated steam for a period of 15 min. There were no statistically significant decreases ($P<0.05$) between the average weights of the birds fed the diets listed in Table 2, but the birds fed the diet containing

guar meal processed for one hour had the best average weights. The chicks fed the raw guar meal processed for 2 hr with the injection of super-heated steam for 15 min. had significantly improved ($P<0.05$) efficiency of food utilisation when compared to that of the other diets.

Experiment 3. The substitution of 20 and 30 per cent commercially processed guar meal for soyabean meal in the diet produced a significant ($P<0.05$) decrease in the weight and food conversion of broiler cross chicks at 25 days of age (Table 3). Chicks fed the diet in which 10 per cent processed guar meal was substituted for an equivalent amount of soyabean meal showed no significant ($P<0.05$) decrease in average weight or food conversion from that of the control birds.

Raw guar meal produced a significant

TABLE 2

Experiment 2: The effect of processing guar meal for ½, 1, 1½ and 2 hr at 110° C. with the injection of super-heated steam at 110° C. on the average weights and efficiency of food utilisation of broiler cross male chicks at 4 weeks of age with the guar meal substituted for soyabean meal at a level of 10 per cent in the diet

Group number	Treatment	Average wt at 4 weeks	Efficiency of food utilisation g. food/g. gain
1	Processing for ½ hr with steam injected for 15 min.	381·2	1·62
2	Processing for 1 hr with steam injected for 15 min.	411·5	1·70
3	Processing for 1½ hr with steam injected for 25 min.	380·4	1·70
4	Processing for 2 hr with steam injected for 15 min.	394·4	1·60
5	Control with soyabean meal	404·4	1·71

Statistical comparison of means (Duncan, 1955)
Average weight at 4 weeks $P<0.05$ 2 5 4 1 3
Efficiency of food utilisation $P<0.05$ 5 2 3 1 4

TABLE 3

Experiment 3: The effect of substituting 10, 20 and 30 per cent commercially processed guar meal (heated for 1 hr with the injection of super-heated steam at temperature of 110° C.) on the average weight and efficiency of food utilisation and pancreas (expressed as percentage of body weight) at 25 days of age

Group number	Treatment	Average weight at 25 days of age (g.)	Mortality (per cent)	Efficiency of food utilisation g. food/g. gain	Pancreas weight expressed as percentage of the body weight
1	Control	400·8	8	2·10	0·45
2	Commercially processed guar meal—10 per cent	390·6	5	2·18	0·43
3	Commercially processed guar meal—20 per cent	294·0	19	2·67	0·48
4	Commercially processed guar meal—30 per cent	173·8	20	3·28	0·55
5	Raw guar meal—10 per cent	227·8	10	2·56	0·52
6	Raw guar meal—20 per cent	219·3	25	2·95	0·49
7	Raw guar meal—30 per cent	80·8	75	3·50	0·57

Statistical comparison of means (Duncan, 1955)
Avg. wt at 4 weeks (g.) $P<0.05$ 1 2 3 5 6 4 7
Efficiency of food utilisation $P<0.05$ 7 4 6 3 5 2 1
Pancreas size $P<0.05$ 7 4 5 6 3 1 2

($P<0.05$) depression in the rate of growth and food conversion when substituted into the basal diet at levels of 10, 20 and 30 per cent (groups 5, 6 and 7) as compared to similar groups where equivalent amounts of commercially processed guar meal was substituted into the basal diet at levels of 10, 20 and 30 per cent respectively (groups 2, 3 and 4). These data illustrate the necessity for heating the guar meal as described in this report for the purpose of destroying the trypsin inhibitor previously reported by Couch, Gregor and Bakshi (1966).

There was no difference in the weight of the pancreas of group 1 (control) and group 2 (10 per cent guar meal). When the level of processed guar meal was increased to 20 and 30 per cent (groups 3 and 4), the pancreas weight expressed as a percentage of the body weight increased to the extent that there was no difference between the pancreas weights of these two groups and those of chicks from groups 5, 6 and 7 which received 10, 20 and 30 per cent raw guar meal respectively. This suggests that there may be another factor in the guar meal which may cause hypotrophy of the pancreas in addition to the trypsin inhibitor.

Experiment 4. The substitution of 2·5 per cent commercially processed guar meal in the chick diet significantly increased the average weights of broiler cross female chicks at 4 and 8 weeks of age and above that of those fed the control diet as well as all other levels of guar meal. From the data there is not any apparent reason for the fact that the substitution of 2·5 per cent guar meal produced a significant increase in the growth rate.

Commercially processed guar meal can be substituted at levels of 2·5, 5, 7·5 and 10 per cent for soyabean meal at the levels indicated without any adverse effect on the growth of the chicks (Table 4). When the

TABLE 4

Experiment 4: The effect of substituting commercially processed guar meal at 2·5, 5, 7·5, 10, 12·5 and 15 per cent for soyabean meal on the average weight and efficiency of food utilisation of female broiler cross chicks at 4 and 8 weeks of age

Group	Treatment	Average wt	Efficiency of food utilisation g. food/g. gain
After 4 weeks			
1	Control	531·3	1·89
2	2·5 per cent guar meal	571·8	2·21
3	5 per cent guar meal	559·1	1·85
4	7·5 per cent guar meal	520·8	2·18
5	10 per cent guar meal	496·8	2·27
6	12·5 per cent guar meal	486·3	2·23
7	15 per cent guar meal	400·2	2·39
After 8 weeks			
1	Control	1268·9	2·53
2	2·5 per cent guar meal	1341·9	2·45
3	5 per cent guar meal	1251·0	2·67
4	7·5 per cent guar meal	1267·6	2·72
5	10 per cent guar meal	1263·1	2·50
6	12·5 per cent guar meal	1151·3	2·68
7	15 per cent guar meal	973·5	2·88

Statistical comparison of means (Duncan, 1955)

Avg. wt at 4 weeks $P<0.05$	2 3 1 4 5 6 7
Avg. wt at 8 weeks $P<0.05$	2 1 4 5 3 6 7
Efficiency of food utilisation to 4 weeks $P<0.05$	7 5 6 2 4 1 3
Efficiency of food utilisation from 4-8 weeks $P<0.05$	7 4 6 3 1 5 2

level of guar meal exceeded 10 per cent, a significant growth depression was observed. The food efficiency figures were somewhat variable in this experiment. The 12·5 and 15 per cent levels of guar meal had an adverse effect on growth and food conversion.

The screens from the bottoms of the cages from the control, those which received 10 per cent guar meal and those which received 15 per cent guar meal were examined. It was apparent that there was a definite stickiness and adherence of the droppings to the screen where the broiler cross female chicks had received a diet containing 15 per cent guar meal. The same was apparent but to a much lesser degree when the birds received 10 per cent guar meal. It was reported earlier (Vohra and Kratzer, 1964*b*) that chick growth was reduced by adding 2 per cent guar gum to the diet and that the droppings of chicks fed 10 per cent guar meal were sticky (Anderson and Warnick, 1964).

Experiment 5. The substitution of 2·7 per cent guar gum or guar splits, the raw material from which the gum is made, for milo in the diet of chicks significantly depressed the growth and efficiency of food utilisation (Table 5). The chicks fed the diet which contained 1·8 per cent guar splits had a growth rate equivalent to those fed 15 per cent guar meal. When fed the diet containing 10 per cent guar meal, the average weights and food efficiencies were not significantly ($P<0·05$) different from that of those fed the soyabean oil meal diet or those which received 1·8 per cent gum. The male and female chicks exhibited similar growth responses to the various treatments.

Experiment 6. The results are given in Table 6. There were significant response differences in growth rate between the chicks fed the various diets. The addition of 0·2 per cent pectinase to the diet which contained 15 per cent guar meal (treatment 3) produced a significant chick growth response above that of those fed the diet which contained 15 per cent guar meal. No significant difference in efficiency of food utilisation existed between the chicks fed the two diets (treatment 2 and 3). The chicks fed the

TABLE 5

Experiment 5: The effect of substituting commercially processed guar meal at the 10 and 15 per cent levels for soyabean meal, guar gum at the 1·8 and 2·7 per cent levels for milo and soyabean meal, and guar splits at the 1·8 and 2·8 per cent levels for milo and soyabean meal on the average weight and efficiency of food utilisation of male and female broiler cross chicks at 4 weeks of age

Group	Treatment	Avg. 4 wk wts (g.) Male	Female	Male & Female	Efficiency of food utilisation g. food/g. gain
1	Control	585·0	531·0	558·0	2·14
2	10 per cent guar meal	573·0	505·0	540·0	2·19
3	15 per cent guar meal	511·5	452·8	482·7	2·23
4	1·8 per cent guar gum[1]	544·8	494·0	519·4	2·34
5	2·7 per cent guar gum	390·0	354·3	372·2	2·84
6	1·8 per cent guar splits[2]	526·0	345·0	480·5	2·62
7	2·7 per cent guar splits	445·7	405·8	425·8	2·95

[1] Jaguar (A -- 20D control 7 -- 5324).
[2] Guar splits (control PG 489, raw material from which guar gum is made) supplied by Stein, Hall and Company, 605, Third Avenue, New York.

Statistical comparison of means (Duncan, 1956)

Male wt $P<0·05$	1 2 4 6 3 7 5
Female wt $P<0·05$	1 2 4 3 6 7 5
Male & Female $P<0·05$	1 2 4 3 6 7 5
Efficiency of food utilisation $P<0·05$	7 5 6 4 3 2 1

TABLE 6

Experiment 6: The effect of adding 0·2 per cent pectinase[1] in the presence of 15 per cent processed guar meal on the average weight and efficiency of food utilisation of broiler cross male chicks at 4 weeks of age

Group	Treatment	Avg. wt at 4 weeks (g.)	Efficiency of food utilisation g. food/g. gain
1	Basal	485·8	1·98
2	15 per cent processed guar meal	351·0	2·24
3	15 per cent processed guar meal+0·2 per cent pectinase	406·2	2·48

[1] Pectinase D, an enzyme preparation supplied through the courtesy of Miles Chemical Company, Elkhart, Indiana.

Statistical comparison of means (Duncan, 1955)

Avg. wt at 4 weeks (g.) $P<0.05$ 1 3 2

Efficiency of food utilisation $P<0.05$ 3 2 1

control ration had a significantly better growth rate and food conversion.

DISCUSSION AND CONCLUSIONS

From the results of this series of experiments, it is apparent that raw guar meal contains a factor which can be destroyed by heat. Such factor has been identified as a trypsin inhibitor (Couch *et al.*, 1966). Best results were obtained in these investigations by heating the guar meal for a period of 1 hr at 110° C. with the injection of super-heated steam after the prescribed temperature had been attained. The trypsin inhibition present in guar meal extracts was almost completely destroyed when the extract was heated in a boiling water bath for 60 min.

The guar meal used in these studies contained approximately 18 per cent guar gum. When used at the 10 per cent level, the diet would contain approximately 1·8 per cent guar gum. From the studies reported herein it is apparent that this level of gum may have an adverse effect on the chick as shown by the stickness of the droppings. It is possible that the detrimental effect of substituting 12·5 and 15 per cent commercially processed guar meal in the chick diet may have been traceable to the gum present in the meal since it was observed that 2·7 per cent guar gum had definite adverse effects on chick growth as well as upon the consistency of the droppings.

REFERENCES

ANDERSON, J. O. AND WARNICK, R. E. (1964). Value of enzyme supplements in rations containing certain legume seed meals and gums. *Poult. Sci.*, 43: 1091-1097.

BORCHERS, R. L. AND ACKERSON, C. W. (1950). The nutritive value of legume seeds × effect of autoclaving and the trypsin inhibitor test for 17 species. *J. Nutr.*, 41: 339-345.

COUCH, J. R., CREGER, C. R. AND BAKSHI, Y. K. (1966). Trypsin inhibitor in guar meal. *Proc. Soc. exp. Biol. Med.*, 123: 263-265.

DUNCAN, D. B. (1955). Multiple range and multiple *F* tests. *Biometrics*, 11: 1-42.

EVERSON, G. AND HECKERT, A. (1944). The biological value of some leguminous sources of protein. *J. Am. diet. Ass.*, 20: 81-86.

HAYWARD, J. W., HALPIN, J. G., HOLMES, C. E., BOHSTEDT, G. AND HART, E. B. (1937). Soybean meal prepared at different temperatures as feed for poultry. *Poult. Sci.*, 16: 3-14.

KUNITZ, M. (1945). Crystallization of trypsin ininhibitor from soybean meal. *Science, N.Y.*, 101: 668-675.

KUNITZ, M. (1946). Crystalline soybean meal trypsin inhibitor. *J. gen. Physiol.*, 29: 149-154.

LYMAN, C. M., HOLLAND, B. R. AND HALE, F. (1944). Processing cottonseed meal. A method for eliminating toxic qualities. *Ind. Engng Chem.*, 36: 188-192.

OSBORNE, T. B. AND MENDEL, L. B. (1917). The use of soybean as food. *J. biol. Chem.*, 32: 369-383.

ORRU, A. AND DAMEL, V. C. (1941). Physiological

and anatomical-pathological observations on rats fed the seeds of *Canavalia ensiferns*. *Chem. Abstr.*, 36: 3532.

RICHARDSON, L. R. (1948). Southern peas and other legume seeds as a source of protein for growth of rats. *J. Nutr.*, 36: 451-462.

SURE, B. AND READ, J. W. (1921). Biological analysis of the seed of Geergier Value Bean. *J. agr. Res.*, 22: 5-8.

VOGT, H. AND PENNER, W. (1963). Die ver Wendung von guar mehl in Geflugimast futter. *Arch. Geflügelk.*, 27: 43.

VAN ETTEN, C. H., MILLER, R. W. AND WOLFF, I. A. (1961). Amino acid composition of twenty-seven selected seed meals. *J. agric. Fd Chem.*, 9: 79-84.

VOHRA, P. AND KRATZER, F. H. (1964*a*). The use of guar meal in chicken rations. *Poult. Sci.*, 43: 502-503.

VOHRA, P. AND KRATZER, F. H. (1964*b*). Growth inhibitor effect of certain polysaccharides for chicken. *Poult. Sci.*, 43: 1164-1170.

WILGUS, H. S., NORRIS, L. C. AND HUESER, G. F. (1936). Effect of heat on the nutritive value of soybean meal. *Ind. Engng Chem.*, 28: 586-588.

3. Geschwind, I. I., Li, C. H., The Hypophyseal Growth Hormone, Nature and Actions, Blakiston Div. of McGraw-Hill, N. Y., 1955, Ch. 3.

4. Asling, C. W., Simpson, M. E., Li, C. H., Evans, H. M., Anat. Rec., 1954, v119, 101.

5. Scow, R. O., Simpson, M. E., Asling, C. W., Li, C. H., Evans, H. M., ibid., 1949, v104, 445.

6. Ray, R. D., Simpson, M. E., Li, C. H., Asling, C. W., Evans, H. M., Am. J. Anat., 1950, v86, 479.

7. Ray, R. D., Asling, C. W., Walker, D. G., Simpson, M. E., Li, C. H., Evans, H. M., J. Bone & Joint Surg., 1954, v36-A, 94.

8. Ray, R. D., Asling, C. W., Simpson, M. E., Evans, H. M., Anat. Rec., 1950, v107, 253.

9. Koneff, A. A., Scow, R. O., Simpson, M. E., Li, C. H., Evans, H. M., ibid., 1949, v104, 465.

10. Koneff, A. A., Van Dyke, D. C., Evans, H. M., Endocrinology, 1952, v51, 249.

11. Contopoulos, A. N., Koneff, A. A., Acta Endocrinol., 1963, v42, 275.

12. Asling, C. W., Simpson, M. E., Evans, H. M., Rev. Suisse de Zool., 1965, v72, 1.

13. Taurog, A., Evans, E. S., Potter, G. D., Chaikoff, I. L., Endocrinology, 1960, v67, 609.

14. Becks, H., Asling, C. W., Collins, D. A., Simpson, M. E., Li, C. H., Evans, H. M., Anat. Rec., 1948, v101, 17.

15. Asling, C. W., Durbin, P. W., Parrott, M. W., Johnston, M. E., Hamilton, J. G., Proc. Soc. Exp. Biol. and Med., 1957, v94, 200.

16. Geschwind, I. I., Li, C. H., Endocrinology, 1958, v63, 449.

17. Narang, G. D., Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1966, v121, 203.

18. Goldman, M., Rosenberg, L. L., LaRoche, G., Srebnik, H. H., Endocrinology, 1966, v78, 889.

19. Rigal, W. M., Radioisotopes and Bone, Blackwell, Oxford, 1962, 197.

20. ———, Proc. Soc. Exp. Biol. and Med., 1954, v117, 794.

Received June 27, 1966. P.S.E.B.M., 1966, v123.

Trypsin Inhibitor in Guar Meal. (31461)

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Cyamopsis tetragonoloba (Guar) is a drought-resistant annual legume which is adapted to a wide range of soil types. The seed has long been used as a source of gum. The residue remaining after extraction of the gum contains 36-45% protein. Attempts to feed this protein concentrate to animals have met with varying degrees of success. Guar meal has been reported to contain toxic factors which have caused diarrhea, a decrease in growth rate, anorexia, and increased mortality(1,2). Guar gum has been reported to decrease growth and increase mortality in studies with chicks(1,2). The meal contains a trypsin inhibitor which could be destroyed by heat, according to preliminary reports from this laboratory(3,4). It was reported earlier (5) that the nutritive value of Guar meal was not improved by autoclaving. The oil or fat of the Guar meal has been reported not to contain a toxic factor(6).

The present report is concerned with the trypsin inhibitor found in Guar meal.

Experimental. An extract of Guar meal was made by placing 20 g of Guar meal in a 250 ml Erlenmeyer flask containing 100 ml of phosphate buffer (pH 7.6, 0.02 M). The flask was shaken gently and allowed to stand in a cold room at 5°C for 6 to 8 hours with intermittent shaking. The mixture was then filtered through Whatman No. 1 filter paper, and the filtrate was used for assay of the toxic factor.

Worthington lyophilized, salt free hemoglobin was prepared according to the method of Anson(7). The procedure of Northrop *et al*(8) was used for the trypsin assay. Tryptic activity was determined at 37°C with the urea-denatured hemoglobin substrate. The extent of proteolysis was determined by reading the absorbance of the 5% trichloroacetic acid soluble degradation products at 280 mμ with a Beckman model DB spectrophotometer.

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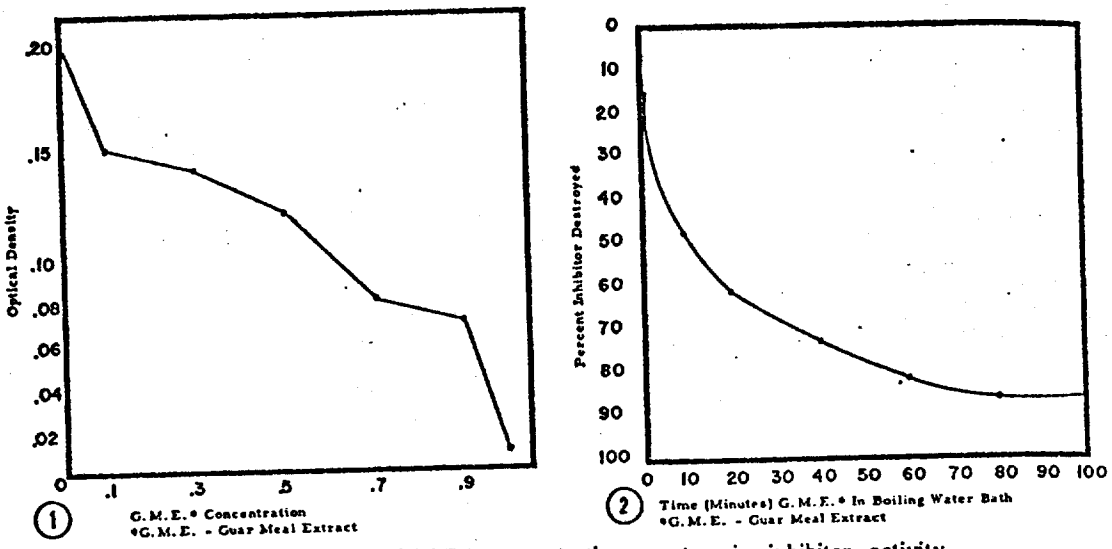

FIG. 1. Effect of G.M.E.* concentration on trypsin inhibitor activity.
FIG. 2. Effect of heat on destruction of G.M.E.* trypsin inhibitor.

For the enzyme assay, 5 ml of the hemoglobin substrate were incubated with 1 ml of trypsin solution for 5 minutes at 37°C. The addition of 10 ml of 5 percent trichloroacetic acid served both to terminate the enzyme action and to precipitate the undigested protein, which was removed by filtration through Whatman No. 3 filter paper. Ten ml of distilled water were added to the 5 ml trichloroacetic acid filtrate. Appropriate blanks were prepared by adding trichloroacetic acid to the substrate prior to addition of enzyme. Substrate and enzyme were equilibrated to the temperature of the water bath (37°C) before being mixed.

In order that the extent of protein hydrolysis would be proportional to the enzyme concentration, the enzyme solution was adjusted to a dilution which would give zero order kinetics during a 5-minute incubation period. For studies on the trypsin inhibitor extracted from Guar meal, cyrstalline trypsin (Armour) solution was added to the Guar meal extract, the mixture was allowed to stand for half an hour, and then assayed for proteolytic activity according to the procedure used for trypsin-hemoglobin preparation. Activity of Guar meal extract was compared with standard trypsin-hemoglobin results to determine the extent of proteolytic activity.

Results and discussion. Guar meal contains a trypsin inhibitor and the level of trypsin inhibition is concentration dependent (Fig. 1). Such has been demonstrated by adding varying levels of the Guar meal extract in the standard trypsin assay procedure of Northrop *et al*(8).

The trypsin inhibitor in Guar meal is destroyed by heating (Fig. 2). When the Guar meal extract was heated for a period of 10 minutes, 50% of the trypsin inhibitor was destroyed. Heating the extract for 60 minutes destroyed 80% of the activity. A continuation of the heating for a period of 80 minutes resulted in some further destruction. It is possible that a more highly purified preparation of the trypsin inhibitor would result in a destruction more nearly approaching 100 %. The fact that the trypsin inhibitor in Guar meal is destroyed by heating contradicts the earlier report(5), and suggests that the trypsin inhibitor is protein in nature.

Dialysis of the Guar meal extract against Sorensens' phosphate buffer (pH 7.6 at 5°C) for periods up to 24 hours and subsequent testing of the dialysate showed that the inhibitor was not dialyzable under these experimental conditions. This also indicated that the inhibitor is a macromolecule.

Summary. The presence of trypsin inhibitor in Guar meal has been demonstrated by the trypsin-hemoglobin digestion procedure. The

level of inhibition is concentration dependent. The inhibitor is destroyed by heating and is apparently not dialyzable, which may be indicative of a macromolecular nature.

1. Vohra, P., Kratzer, F. H., Poultry Sci., 1964, v43, 1164.
2. Anderson, J. O., Warnick, R. E., ibid., 1964, v43, 1091.
3. Bakshi, Y. K., Couch, J. R., Creger, C. R., ibid., 1964, v43, 1302.
4. Couch, J. R., Bakshi, Y. K., Prescott, J. M., Creger, C. R., Fed. Proc., 1965, v24, 687.
5. Borchers, R., Ackerson, C. W., J. Nutrition, 1950, v41, 339.
6. Dijkstra, N. D., Landbouwkundig Tijdschrift, 1962, v74, 822.
7. Anson, M. L., J. Gen. Phys., 1938, v22, 79.
8. Northrop, J. H., Kunitz, M., Herriott, R. M., Crystalline Enzymes, Columbia Univ. Press, New York, 1948, Chap. VI, p125.

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Termination of Immunologic Tolerance in Mice by Antigen-Antibody Complexes.* (31462)

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The theory of antibody formation of Eisen and Karush(1) proposes an extracellular control of tolerance and immunity exerted by circulating antibodies. According to Eisen and Karush, the ratio of the concentrations of antigen and antibody (natural or immune) determines whether the outcome of the exposure of an animal to an antigen will be immunity or tolerance. Thus, it should be possible to terminate immunologic tolerance by manipulations intended to shift the ratio of antigen to antibody from one giving rise to complexes of 2 molecules of antigen with one of antibody (Ag_2Ab) assumed by the model to result in tolerance, to one favoring the formation of complexes of one molecule of antigen with one of antibody (AgAb) which, according to the model, will induce immunity. Since the Ag_2Ab complexes are assumed to be unable to gain entrance into the immunologically competent cells, a specific prediction of this model is that tolerant animals should contain cells immunologically competent to respond to the tolerated antigen. Because other theories of antibody formation explain tolerance by the absence or the repression of immunologically competent cells genetically capable of synthesizing antibody specific to the tolerated antigen, the demonstration of the existence of inducible cells in lymphoid tissues of a tolerant animal, and their induction by bimolecular complexes of antigen and antibody, would provide strong support for an extracellular control of tolerance and immunity of the type postulated by Eisen and Karush. Successful attempts to terminate tolerance by the means outlined are described here.

Materials and methods. Fifty-four NIH strain white mice‡ received 3 subcutaneous injections of 20 mg of crystallized human serum albumin (HSA)§ each on the day of birth and at 2 and 3 weeks of age. A fourth injection of 20 mg HSA was given intraperitoneally at 6 weeks of age to insure maintenance of the tolerant state. At 6 weeks of age the mice also received 20 μg of radioiodinated human serum albumin (I^{131} HSA).‖

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† Captain, U. S. Air Force, assigned to Univ. of Illinois under sponsorship of Air Force Inst. of Technology. Present address: U. S. Army Biological Center, Fort Detrick, Maryland.

‡ Adult mice were obtained from Belair Acres Laboratory Animals, Danville, Ind., and bred at the authors' laboratory.

§ Pentex, Inc., Kankakee, Ill.

‖ Abbott Laboratories, North Chicago, Ill. I^{131} HSA had a specific activity of approximately 10 μc/mg and was used within 48 hours of receipt.

APPLICATION OF THE CHROMIC OXIDE INDICATOR METHOD TO BALANCE STUDIES WITH GROWING CHICKENS

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TWO FIGURES

(Received for publication February 18, 1952)

The determination of a nutrient balance is a basic technique which has been applied extensively in nutritional studies of digestibility, utilization and retention. The procedure most frequently followed is to collect all the excrement produced during the experimental period and, by comparison with the total food intake, to determine the "disappearance" of the nutrient under study. This procedure is laborious and time-consuming.

The basic data needed to establish a balance comprise the amounts of excrement derived from the measured food intake over a period of time sufficiently long to give a representative sample characteristic of the experimental conditions employed. The procedure can be facilitated by any method which will aid in establishing the amount of excrement equivalent to a given amount of food. In one such method, a known amount of a completely indigestible index material is incorporated in the diet; the amount of food from which a sample of feces was derived can be calculated from the respective concentrations of index material in food and feces. Chromic oxide (Edin, '18; Edin et al., '44; Schürch et al., '50), iron oxide (Bergeim, '26), barium sulfate (Whitson et al., '43) and lignin (Kane et al., '50) have been employed as index substances.

Less work in this field has been done with poultry than with other species. Determination of true digestibility with birds is complicated by the fact that urinary and fecal excrements are voided together.

The studies which have been made are typified by those of Whitson et al. ('43) and Olsson ('50). The former workers used barium sulfate as the index material in determining fat utilization by growing chickens. Olsson applied the "Edin Indicator Method" to digestibility studies with mature hens, geese, and turkeys on restricted dietary regimes. In both cases the balance experiments were carried out on birds kept in individual cages.

The insoluble nature of chromic oxide and the practicability of its use as an index substance have been well established in the studies already referred to. The purpose of the present work was to define the conditions of its applicability to balance studies with groups of growing chicks under ad libitum feeding conditions, with particular reference to the following: (1) Length of collection period necessary to assure a representative sample of excrement; (2) reproducibility of results in successive collection periods; (3) recovery of ingested chromic oxide, and (4) comparison of balance data by the index and total collection methods.

In the course of these studies the colorimetric method of Schürch and co-workers ('50) for determining chromium was modified to improve its sensitivity.

EXPERIMENTAL PROCEDURE AND RESULTS

Analytical method

Colorimetric analysis of chromium, first developed by Paloheimo and Paloheimo ('35) and modified by Schürch et al. ('50), has the advantages over other methods of speed and simplicity, so desirable in a routine method to be applied to a large number of samples. The method used in this laboratory is essentially that of Schürch and co-workers except for the measurement of color density. The sample of diet or excre-

ment is ashed, and the ash fused with sodium peroxide to convert chromic oxide to chromate. After appropriate dilution, the color density is measured in a Beckman DU spectrophotometer. Determination of the absorption spectrum of

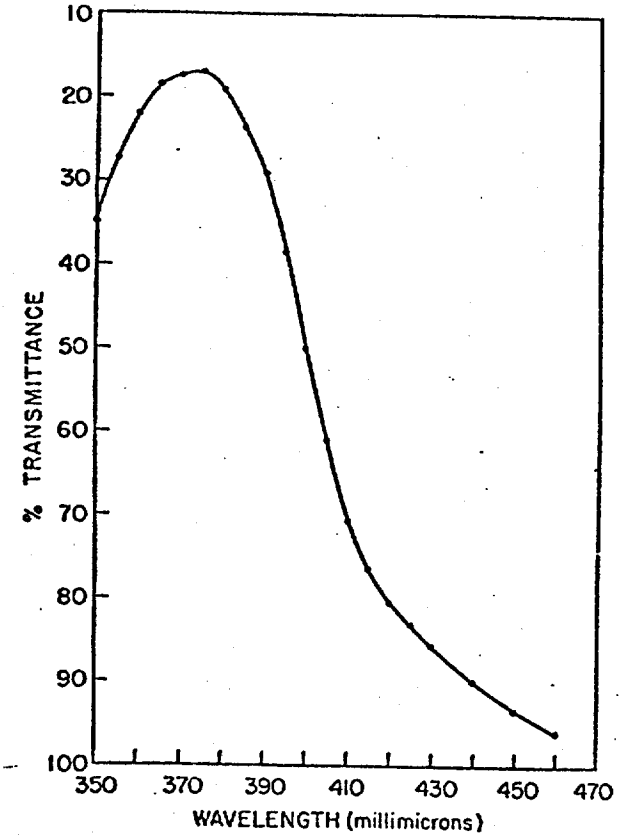

Fig. 1 Absorption spectrum of chromate from fused chromic oxide (concentration equivalent to 12 μg chromic oxide per milliliter).

such a solution prepared from fused chromic oxide showed a single absorption maximum at 375 mμ (fig. 1). A standard reference curve was established at this wavelength by determining the optical density of solutions prepared from known amounts of chromic oxide carried through the fusion process. Figure 2 shows the linear relation between the optical density

of the chromate solution and the concentration of chromium expressed as the amount of chromic oxide prior to fusion the linear regression was derived statistically from data based on three or more replicate determinations at each of 7

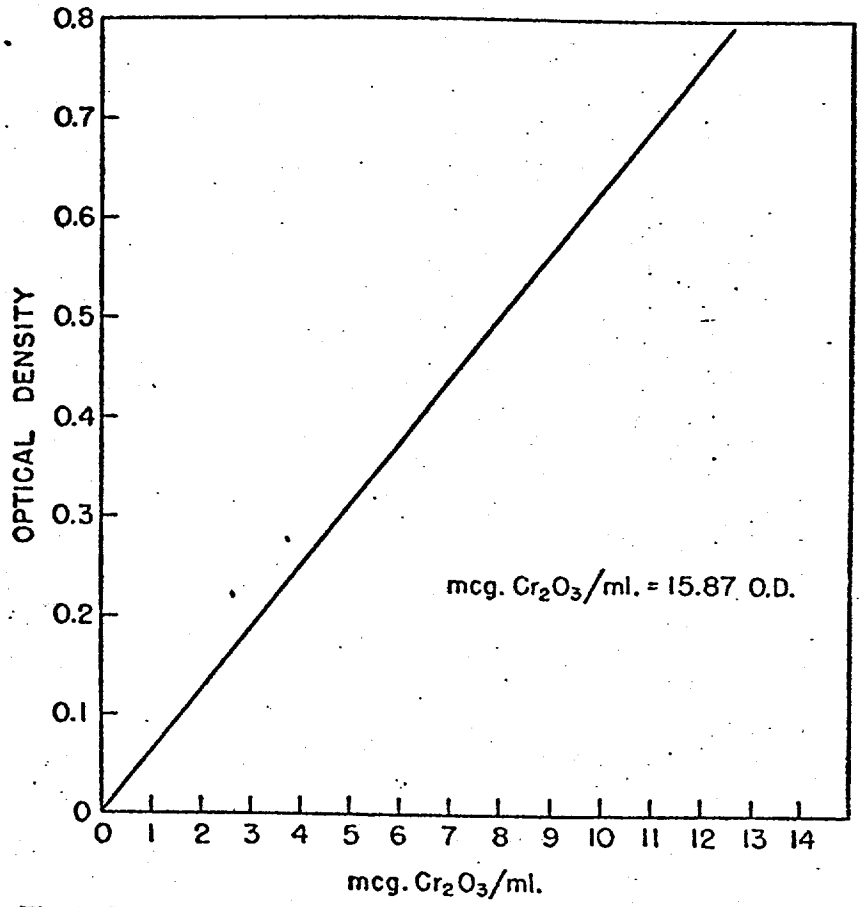

Fig. 2 Linear relation at 375 mμ between optical density and chromium content of solutions derived from fused chromic oxide.

chromium concentrations. In contrast to the results reported by Schürch and co-workers, determining the color density at 375 mμ gives excellent conformity with the Lambert-Beer law, and also increases the sensitivity of the method materially. The standard deviation of a single determination, derived

from the data used to establish the reference curve, was found to be ± 0.11 μg chromic oxide per milliliter for concentrations of up to 12 μg per milliliter. Precision tended to decrease above this concentration, as indicated by a disproportionate increase in standard deviation, possibly due to deviation from the Lambert-Beer law.

Feeding experiments

Chromic oxide was incorporated in the experimental diets either as the pure powder at a 1% level (experiment I), or as a chromic oxide-flour-water bread prepared as suggested by Kane and co-workers ('50) and finely ground in a Wiley mill. The latter procedure was employed in experiments II and III, using a level of chromic oxide bread sufficient to supply 0.2% chromic oxide in the diet.

The ration used in experiment I was composed of natural feedstuffs and was typical of rations commonly used for young chicks under farm conditions. A more highly digestible semi-purified ration was used in experiments II and III. It contained cerelose and casein in addition to ground corn and wheat, soybean flour, fish meal, yeast, and mineral and vitamin supplements. Except for the higher digestibility of the latter ration, which will be evident in the data presented, the results were not influenced by the nature of the rations.

Groups of 10 to 20 chicks between 4 and 5 weeks of age were used in these studies, housed in cages with wire floors 3.5 by 4.5 feet in size. The diets containing chromic oxide were fed for a three-day preliminary period, after which time collections of excrement were made, using galvanized sheet metal trays suspended beneath the cages. Daily measurements of feed consumption were made and particular care was taken to account for all feed wastage by attaching shallow screened trays beneath the feeders to catch feed spilled by the chicks. This procedure also effectively minimized contamination of excrement with feed.

Collections of excrement were made as quantitatively as the conditions permitted at intervals of 8 to 24 hours, with

arrangement of periods such that the data could be groupe into successive 24-hour periods. The excreta were froze immediately after collection and dried in the frozen state i a Dessivac apparatus.

In theory, a small sample of excrement would be sufficie to provide the data for calculating the retention or disappea ance of a nutrient. The physiological processes of the chicke do not result in the production of excrement of uniform com position throughout a 24-hour period, however, primaril because of diurnal rhythm in the elimination of cecal drop pings which contain only traces of urinary excreta (Herric and Edgar, '47). Collections during periods of less than 1

TABLE 1

Chromic oxide content of excrement in successive 24-hour periods

COLLECTION PERIOD	CHROMIC OXIDE CONTENT Experiment		
	I	II	III
	%	%	%
1	2.70	1.04	1.11
2	2.60	1.03	1.03
3	2.56	0.84	1.00
4		1.05	1.17

hours showed excessive variability of chromium content; to conserve space, these data are not reported in detail. Instead the data have been combined into 24-hour periods.

Table 1 shows the chromic oxide content of the excreta on a moisture-free basis for successive 24-hour periods in the three experiments. The precision of the colorimetric deter- mination of chromium is such that practically all of the varia- tions from period to period can be considered to be due to variability among the animals and to feeding conditions. In the main the data show excellent agreement in successive collection periods, indicating that 24-hour periods are satis- factory units for this type of work.

The two exceptions, 0.84% in experiment II and 1.17% in experiment III, are probably due to changes in level of feed

intake, as shown in table 2. The value of 0.84% in experiment II was obtained during a 24-hour period of elevated feed intake and increased output of excreta. The reasons for the change in consumption were not evident, but since a temporarily higher plane of nutrition was produced it would be expected that digestive efficiency would be somewhat lower. Conversely, in experiment III the high value of 1.17% chromic oxide occurred in a period when feed intake was purposely reduced and production of excreta was low. This temporarily lowered plane of nutrition was accompanied by an increased

TABLE 2

The effect of feed intake on output of feces and fecal level of chromic oxide

COLLECTION PERIOD	FEED INTAKE	FECES OUTPUT	Cr_2O_3 IN FECES
	gm	*gm*	%
	Experiment II		
1	1,056	244	1.04
2	1,137	243	1.03
3	1,191	288	0.84
4	1,155	258	1.05
	Experiment III		
1	1,067	240	1.11
2	1,140	240	1.03
3	1,158	252	1.00
4	941	148	1.17

efficiency of utilization of the diet. These exceptions to the general uniformity of fecal levels of chromic oxide show that a single collection period may not produce a representative excrement sample. Agreement in the chromic oxide content of two or more successive 24-hour collections of excrement can be used as a criterion of the uniformity of conditions and the validity of the balance measurement.

Data are presented in table 3 showing the recovery of ingested chromic oxide in the feces. Large fluctuations between periods were observed, but over the entire three or 4 days of each experiment approximately 95% of the ingested chromic

oxide was recovered in the excrement. Recovery for experiment III was 96% when the low value for period 4 was omitted from the calculations; omission of this period is probably justified for the reasons discussed above. Incomplete recovery of ingested chromic oxide could have been due to (1) incomplete collection of feces, caused in part by the large size of the cages used, and (2) the somewhat larger capacity of the digestive tract of the rapidly growing birds at the end of the period as compared to the beginning. Recovery of 99% of the chromic oxide ingested by hens maintained in special individual cages has been reported by Olsson ('50). Failure

TABLE 3

Recovery of ingested chromic oxide in successive 24-hour periods

COLLECTION PERIOD	RECOVERY OF CHROMIC OXIDE Experiment		
	I	II	III
	%	%	%
1	91	102	105
2	127	94	91
3	76	87	91
4		100	77
Total recovery	94	95	92

to obtain this degree of recovery in the present work was probably due mainly to the difficulty of achieving quantitative collection of excrement under the conditions employed. More striking than the extent of recovery over the entire period were the extreme daily variations, due probably to the fact that fecal output during a given 24-hour period does not represent the feed consumed in the same period. These data demonstrate a major advantage of the index method over the method of total collection in studies covering relatively short time intervals.

The results of applying the chromic oxide data to calculation of dry matter utilization and nitrogen retention are

shown in tables 4 and 5 in comparison with the balances determined by measurement of the total intake and excretion. With the exceptions noted above, the successive 24-hour collections showed uniform utilization values. Comparison of the average values determined by the index method with the values determined by measuring total intake and excretion shows a higher degree of utilization found by the latter method in every case. This is probably due to the fact that any failure to achieve quantitative collection of excrement would result in the finding of a higher apparent degree of utilization by the

TABLE 4

Dry matter utilization determined by index method in comparison to total measurement of intake and excretion

COLLECTION PERIOD	UTILIZATION OF DRY MATTER BY INDEX METHOD Experiment		
	I	II	III
	%	%	%
1	61.9	77.5	78.6
2	60.4	77.3	76.9
3	59.8	72.1	76.2
4		77.7	79.7
Average	60.7	76.2	77.8
Calculated from total measurement	63.1	77.3	79.6

total collection method, but would not affect the results obtained by the index method. The discrepancy is of the same order of magnitude as the amount of ingested chromic oxide unaccounted for in the total balance (table 3). As would be expected from the data in table 3, calculation of the utilization of dry matter and nitrogen for 24-hour periods by the total collection method gave highly variable results for successive periods, in contrast to the relatively uniform results obtained by the index method.

Nitrogen retention values calculated by the index method were lower than those determined by the total collection method to an extent greater than the apparent loss of in-

gested chromic oxide. This suggests that the excreta lost b adherence to the screen floors was relatively richer in chromi oxide than nitrogen, with the result that the nitrogen excre tion per unit of chromic oxide was erroneously high and th nitrogen retention correspondingly low. This could occur i cecal droppings were preferentially lost. Their physical na ture is such that they would tend to adhere to the screen floor more readily than normal droppings. This explanation i

TABLE 5

Nitrogen retention determined by index method in comparison to total measurement of intake and excretion

COLLECTION PERIOD	NITROGEN RETAINED BY INDEX METHOD Experiment	
	II	III
	%	%
1	48.02	47.26
2	47.29	40.61
3	37.53	42.88
4	45.84	50.17
Average	44.67	45.23
Calculated from total measurement	47.26	49.66

consistent with the observation that the dry matter balanc by the two methods of determination disagreed less tha would be expected by the extent of loss of the ingested chromi oxide.

SUMMARY

The colorimetric method of Schürch and co-workers fo the determination of chromium has been modified by measur ment of color density at 375 mμ. With this modification, th method is highly sensitive and conforms to the Lambert-Be law.

The index method of conducting measurements of nutrie balance using chromic oxide has been applied to groups growing chickens under ad libitum feeding conditions.

Collecting excrement in unit periods of 24 hours is satisfactory for circumventing the effect of the diurnal rhythm of excretion of cecal droppings. Changes in level of feed intake are reflected quickly in the level of chromic oxide in the excrement, with the result that any single 24-hour collection may be subject to serious error. A useful criterion for determining the validity of a balance obtained by this method is the agreement between two or more successive 24-hour periods in the chromic oxide content of the excrement.

Compared to results obtained by measurement of total intake and excretion, the index method gave more consistent and probably more accurate results of balance measurements under the conditions employed.

LITERATURE CITED

BERGLIM, O. 1926 Intestinal chemistry. IV. A method for the study of food utilization or digestion. J. Biol. Chem., *70:* 29.

EDIN, H. 1918 Orienterande försök över användbarheten av en på "ledkroppsprincipen" grundad metod att bestämma en foderblandnings smältbarhet. Centralanstalten för försöksväsendet på jordbruksområdet. Stockholm Medd. Nr., *165:* 1–28.

EDIN, H., G. KIHLEN AND S. NORDFELDT 1944 A summarized description of "Edin's Indicator Method" for the determination of the digestibility of feeds and feed mixtures. Ann. Agr. Coll. Sweden, *12:* 166.

HERRICK, C. A., AND S. A. EDGAR 1947 Some relationships between cecal function and coccidiosis of chickens. Poultry Sci., *26:* 105.

KANE, E. A., W. C. JACOBSON AND L. A. MOORE 1950 A comparison of techniques used in digestibility studies with dairy cattle. J. Nutrition, *41:* 583.

OLSSON, N. 1950 Smältbarhetsförsök med Fjäderfä. Kungl. Lantbrukshögskolan och Statens Lantbruksförsök Statens Husdjursförsök. Stockholm Medd. Nr., *43:* 1–69.

PALOHEIMO, L., AND I. PALOHEIMO 1935 Eine photometrische Methode zur Bestimmung des Cr_2O_3 bei Verdaulichkeitsuntersuchungen nach sog. quantitativen Indikatorverfahren. Tierernährung, *7:* 317–324.

SCHÜRCH, A. F., L. E. LLOYD AND E. W. CRAMPTON 1950 The use of chromic oxide as an index for determining the digestibility of a diet. J. Nutrition, *41:* 629.

WHITSON, D., C. W. CARRICK, R. E. ROBERTS AND S. M. HAUGE 1943 Utilization of fat by chickens — a method for determining the absorption of nutrients. Poultry Sci., *22:* 137.

5. Hohmann, T. C., Goodgold, J., *Am. J. Phys. Med.*, 1961, v40, 52.

6. French, J. H., Clark, D. B., Butler, H. G., Teasdall, R. D., *J. Pediat.*, 1961, v58, 17.

7. Hodes, R., Larrabee, M. G., German, W., *Arch. Neurol. and Psychiat.*, 1948, v60, 340.

Received April 13, 1962. P.S.E.B.M., 1962, v110.

Effects of Gum Guar, Locust Bean Gum and Carrageenan on Liver Cholesterol of Cholesterol-Fed Rats.* (27585)

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Our previous studies(1) indicate that pectin N.F. when fed at a 5% level in the diet largely counteracted the increment in liver cholesterol and liver total lipids induced by cholesterol feeding in the rat. Other roughage or bulk-forming materials such as cellulose, agar, sodium alginate, protopectin and calcium silicate (Micro-Cel) were ineffective in this regard. Data are presented here indicating that Gum Guar, Locust Bean Gum and carrageenan also have significant activity in counteracting the increment in liver cholesterol and liver total lipids induced by cholesterol feeding in the rat.

Procedure. The basal ration consisted of sucrose, 61%; casein,† 24%; cottonseed oil, 10%; salt mixture,‡ 5%; and the following vitamins per kg of diet: thiamine hydrochloride, 20 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; p-aminobenzoic acid, 400 mg; inositol, 800 mg; 2-methyl-1,4 naphthoquinone, 5 mg; vit B_{12}, 150 μg; choline chloride, 2 g; vit A, 5000 U.S.P. units; vit D_2, 500 U.S.P. units; and alpha-tocopheryl acetate, 100 mg. Tests were conducted with rats fed the basal ration, the basal ration + 1% cholesterol, and the basal ration + 1% cholesterol + the various supplements indicated in Table I. The cholesterol and test supplements were incorporated in the basal ration in place of an equal amount of sucrose. Fifty-six male rats of the Holtzman strain with an average body wt of 43.6 g (range 38 to 50 g) were divided into 7 comparable groups of 8 each, placed in metal cages with raised screen bottoms (2 or 3 rats per cage), and provided the test diets and water *ad libitum*. Animals were fed on alternate days and all food not consumed 48 hours after feeding was discarded. Body weights were recorded weekly. After 28 days of feeding, the rats were anesthetized with sodium pentobarbital, and blood was withdrawn from the aorta into a heparinized syringe. Livers were excised, blotted to remove excess blood, weighed and stored in a freezer until analyzed. Lipid was extracted from the livers by the method of Thompson *et al.*(2), and cholesterol was determined on liver and plasma by the method of Nieft and Deuel(3). Total lipids were determined gravimetrically on an aliquot of the liver extract.

Results. The increment in liver cholesterol and liver total lipid induced by cholesterol feeding in the rat was largely counteracted by the concurrent feeding of Gum Guar, Locust Bean Gum or carrageenan at a 10% level in the diet. The effects of these supplements were similar to, although slightly less marked than, that of a comparable amount of pectin N.F. Cholesterol feeding also induced a slight increment in plasma cholesterol levels which was partially counteracted by concurrent feeding of pectin N.F. and several of the other test supplements. Differences between the various groups however were not statis-

* This investigation was supported in part by a research grant from Nat. Heart Inst., N.I.H., P.H.S.

† Vitamin-Free Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Hubbell, Mendel and Wakeman Salt Mixture, General Biochemicals, Inc., Chagrin Falls, Ohio.

TABLE I. Effects of Dietary Supplements on Plasma and Liver Cholesterol and Liver Total Lipid of Cholesterol-Fed Rats (8 Animals/Group).*

Supplements fed with basal ration	Body wt at sacrifice, g†	Plasma cholesterol, mg/100 ml†		Liver cholesterol, mg/g†		Liver total lipid, %†
		Free	Total	Free	Total	
None	197.4 ± 4.9	20.1 ± 1.6	85.2 ± 5.8	1.63 ±.09	2.09 ± .09	4.74 ±.3
1% cholesterol	201.3 ± 6.8	21.0 ± 1.1	105.5 ± 5.0	2.72 ±.14	16.17 ±1.42	9.03 ±.7
Idem + following supplements:						
5% pectin N.F.	185.4 ± 6.1	19.6 ± 1.0	92.8 ± 4.2	2.45 ±.13	5.59 ± .64	6.31 ±.4
10% " "	181.7 ± 5.6	18.2 ± 1.4	95.6 ± 3.3	2.38 ±.17	4.12 ± .42	4.78 ±.3
10% Gum Guar	177.6 ± 5.2	20.8 ± 1.2	91.3 ± 4.3	2.29 ±.12	6.05 ± .79	6.29 ±.5
10% Locust Bean Gum	180.3 ± 5.4	24.0 ± 1.5	103.3 ± 5.0	2.38 ±.08	7.59 ± .73	6.81 ±.4
10% carrageenan	191.9 ± 3.8	20.8 ± 1.6	89.3 ± 5.5	2.22 ±.09	5.52 ± .62	5.86 ±.3

* Test supplements obtained from the following sources: pectin N.F. (citrus), Sunkist Growers, Ontario, Calif.; Gum Guar and Locust Bean Gum, Hathaway Allied Products, Los Angeles, Calif.; carrageenan (Gelcarin MR 100), Marine Colloids, Inc., of America, New York. Test supplements were all natural sources of hydrophyllic complex carbohydrate colloids made up of repeating units of (1) galacturonic acid in the case of pectin, (2) mannose and galactose in Gum Guar and Locust Bean Gum, and (3) sulphated galactose in Gelcarin MR 100. The pectin N.F. employed was a purified material obtained from the dilute acid extract of the inner portion of the rind of citrus fruits. It had a methoxyl value of 10.7% on a moisture-ash-free basis. Gum Guar was the ground endosperm of Guar (known botanically as *Cyamopsis tetragonolaba*) seed with a galactomannan content of approximately 80%. Locust Bean Gum was obtained from the endosperm of the kernels of the Carob tree. It was a hemicellulose product of about 4 parts mannose and 1 part galactose. Gelcarin MR 100, a highly purified product designed for use in milk or milk products, is known chemically as carrageenan. The latter occurs naturally in a number of red seaweeds (class Rhodophyceae) but is obtained principally from the group of seaweeds known as Irish moss.

† Including stand. error of mean.

tically significant.§ Findings are summarized in Table I.

Discussion. A number of studies indicate that, in general, populations habitually subsisting on diets low in fats and animal protein tend to have a low concentration of serum cholesterol and a low incidence of cardiovascular disease(4-7). Such diets contain a number of constituents which are either absent from or present in only minute amounts in the diets of populations with a high incidence of hypercholesterolemia, atherosclerosis and coronary artery disease. Walker and Arvidsson(8) and Higginson and Pepler(9) were among the first to call attention to the high fiber content of the Bantu diet as a possible explanation for the low serum cholesterol level observed in the Bantu population. A similar suggestion was also made by Bersohn *et al.*(10). More recently Keys *et al.* (11) conducted controlled experiments in which groups of physically healthy men subsisted alternately on "American" and "Italian" types of diets, devised to be comparable in calories, proteins and in kind and amounts of fat but differing in the sources of carbohydrates. An abundance of fruits and vegetables in the Italian type diets (which tended to be high in complex carbohydrates such as pectins, hemicelluloses and fiber) replaced equivalent calories in simpler carbohydrates in the American type. Serum cholesterol lev-

§ Food intake was determined for rats in the various groups. Since the weight increment of rats fed the test supplements was less than that of rats fed the basal + cholesterol diet, the question arose whether the reduction in weight increment of rats fed the test supplements may not have been due to a reduction in amount of diet and hence cholesterol ingested and whether the reduced cholesterol intake in turn may not have been the cause of lower liver cholesterol and liver total lipid levels. Such, however, does not appear to be the case, for differences in body weight between the various groups were not statistically significant whereas differences in liver cholesterol and liver total lipid were. Furthermore, an even greater reduction in body weight was observed (unpublished findings) in rats fed the basal ration + cholesterol + 20% alfalfa meal without an accompanying reduction in liver cholesterol and liver total lipid values. The possibility that the test supplements inhibited absorption of cholesterol from the gut, however, has not been excluded.

els were significantly lower with the "Italian" type diets. In subsequent studies Keys *et al.* (12) reported that citrus pectin when fed at a level of 15 g per day caused a slight but statistically significant reduction in serum cholesterol levels in physically healthy, middle-aged men; cellulose (fiber) fed under comparable conditions was without significant effect. These studies suggest that cellulose or fiber *per se* was not responsible for the low serum cholesterol levels of the Bantu and comparable groups, but that pectin, another complex bulk-forming carbohydrate, may have had some activity in this regard.‖ An analysis of the diets of native populations with low serum cholesterol levels indicated that in addition to pectin such rations also contain gums and/or other complex carbohydrates such as colloids of marine plants which were found in the present experiment to cause a highly significant reduction in liver cholesterol levels in the cholesterol-fed rat. Further studies are indicated to determine what effect these substances might have, when administered alone or in combination with one another and pectin, in treatment of hypercholesterolemia and atherosclerosis in man.

Summary. The increment in liver cholesterol and liver total lipid induced by cholesterol feeding in the rat was largely counteracted by concurrent feeding of Gum Guar, Locust Bean Gum or carrageenan at a 10% level in the diet. Effects were similar to, although slightly less marked, than that obtained with a comparable amount of pectin N.F.

‖ Unpublished studies from this laboratory indicate that different batches of pectin may vary markedly in anti-cholesterol activity. Pectic preparations with a methoxyl content of 5% or less were without activity in counteracting the increment in plasma and liver cholesterol levels induced by cholesterol feeding in the rat in contrast to the marked activity exhibited by pectin N.F. preparations of relatively high methoxyl content (10.7% on a moisture-ash-free basis).

1. Wells, A. F., Ershoff, B. H., *J. Nutrition*, 1961, v74. 87.
2. Thompson, S. Y., Ganguly, J., Kon, S. K., *Brit. J. Nutrition*, 1949, v3, 50.
3. Nieft, M. L., Deuel, H. J., Jr., *J. Biol. Chem.*, 1949, v177, 143.
4. Keys, A., Anderson, J. T., *Symposium on Atherosclerosis, Nat. Acad. Sci.*, Nat. Res. Council Publ. 338, 1954.
5. Keys, A., Anderson, J. T., Fidanza, F., Keys, M. H., Swahn, B., *Clin. Chem.*, 1955, v1, 34.
6. Keys, A., *J. Am. Med. Assn.*, 1957, v164, 1912.
7. Yudkin, J., *Lancet*, 1957, v2, 155.
8. Walker, A. R. P., Arvidsson, U. B., *J. Clin. Invest.*, 1954, v33, 1358.
9. Higginson, J., Pepler, W. J., *ibid.*, 1954, v33, 1366.
10. Bersohn, I., Walker, A. R. P., Higginson, J., *S. African M. J.*, 1956, v30, 411.
11. Keys, A., Anderson, J. T., Grande, F., *J. Nutrition*, 1960, v70, 257.
12. Keys, A., Grande, F., Anderson, J. T., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 555.

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Sequential Alterations of Lactic Dehydrogenase Isozymes During Embryonic Development and in Tissue Culture.* (27586)

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Enzymatic changes during growth and development have been extensively studied. The early chick embryo contains low phosphatase activity(1) relatively high peptidase activity(2) and very high concentrations of cytochrome oxidase and succinoxidase(3). During fetal development sequential alterations of phenylalanine hydroxylase, tyrosine transaminase and phenylalanine transaminase in rabbits(4) and of arginase in chicks(5), have been described, and each of these enzymes exhibits a characteristic pattern of

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GUMS, NATURAL

Plant gums and mucilages have been known and used in industry and commerce for thousands of years in various parts of the world. The ancient Egyptians used gums as glue for coating linens employed in clothing corpses for embalming, and also utilized gum arabic as an adhesive for mineral pigments in paint formulations. Historical records likewise show the utilization of various seaweed gums for food and medicinal purposes by many coastal inhabitants of Africa, Asia, Australia, as well as western Europe (1).

Originally the term "gum" was probably applied to natural plant exudates which had oozed from tree barks and hardened upon exposure to air. This designation also covers water-insoluble resins, rubber, latex, and chicle, as well as water-soluble substances. This semantic ambiguity has persisted down to the present time and even today many of the water-insoluble resins used in the paint and chemical industry are incorrectly referred to as "gums." With the advancement of science and technology, an effort has been made to standardize and clarify the meaning of this word.

Today the accepted technical definition of "gum" refers to any material that can be dissolved or dispersed in water to give viscous or mucilaginous solutions or dispersions. This criterion of water solubility covers the large majority of gum materials encountered in industry. The artificial distinction between "gums" and "mucilages" has been eliminated since it was found that the category of "mucilages" had no chemical significance and only served to confuse the issue (2).

The term "gum" is used in industry today to include plant mucilages, and generally refers to plant materials and derivatives which are dispersible in hot or cold water to produce viscous mixtures or solutions. Included in this category are the water-soluble or water-swellable derivatives of cellulose and starch, and the derivatives and modifications of other polysaccharides which are insoluble in the natural form. Included are also certain protein substances, such as gelatin and casein and their deriva-

Table 1: Classification of Gums

Natural	Modified (semisynthetic)	Synthetic
plant exudates	cellulose derivatives	vinyl polymers
arabic	carboxymethylcellulose	polyvinylpyrrolidone
tragacanth	methylcellulose	polyvinyl alcohol
karaya	hydroxypropylmethylcellulose	carboxyvinyl polymer
ghatti	hydroxypropylcellulose	acrylic polymers
seed or root	hydroxyethylcellulose	polyacrylic acid
locust bean	ethylhydroxyethylcellulose	polyacrylamide
guar	starch derivatives	ethylene oxide polymers
psyllium seed	carboxymethylstarch	
quince seed	hydroxyethylstarch	
seaweed extracts	hydroxypropylstarch	
agar	microbial fermentation gums	
algin	dextran	
carrageenan	polysaccharide B-1459	
furcellaran	others	
others	low-methoxyl pectin	
pectin	propylene glycol alginate	
gelatin	triethanolamine alginate	
starch	carboxymethyl locust bean gum	
larch gum	carboxymethyl guar gum	

tives, which have the properties of water solubility and viscosity. In addition the creation of new organic polymers has yielded a whole group of new, completely synthetic gums, such as vinyl polymers, acrylic acid polymers, and ethylene oxide polymers. In the future, many new, tailor-made gums designed for specific applications will undoubtedly be synthesized and will probably replace many natural gums.

A convenient classification scheme consists, therefore, of the following three major groups of gums:

1. Natural gums—found in a natural state, such as the tree exudates or seaweed hydrocolloids.

2. Modified (semisynthetic) gums—chemically modified natural gums or derivatives of naturally occurring materials, such as cellulose or starch.

3. Synthetic gums—completely synthesized chemical products, such as polyvinylpyrrolidone or ethylene oxide polymers.

Table 1 lists the more common gums used in industry today. Many other gums are known and are commercially available, but are not important enough to be included in a general discussion. This article will discuss the important natural plant gums, including the tree exudates, and the seed and root gums. See also Hydrocolloids; Seaweed colloids.

Economics

The utilization and true value of the natural gums in industry is difficult to ascertain, mainly because many of the applications are trade secrets and are not widely publicized. However, since most of the gums are imported and as such are recorded by the U.S. Department of Commerce, reasonable estimates as to quantity and value may be made. Figures for 1963, shown in Table 2, indicate an annual consumption of 71,000,000 lb of natural gums with a market value of about $30,000,000 (3). If the common seaweed gums (agar, algin, carrageenan, and furcellaran) were also included, this would add up to a total of about 90,000,000 lb with a market value of approx $50,000,000. The market for these natural gums has been increasing steadily despite pressures from synthetic products, and it is believed that, as additional applications for gums are found, the markets for both natural and synthetic gums will grow simultaneously.

Table 2. Market Values and Major Applications of Natural Gums (3)

Gum	$/lb	Value[a]	Major applications	Quantity[b]
arabic	0.25	5.2	beer, confectionery, lithography	21.0
ghatti	0.47	0.4	wax emulsions	0.8
guar	0.30	7.8	paper, drilling muds, ore flotation	26.0
karaya	0.51	5.1	denture powders, laxatives, textiles, paper	10.0
larch	0.75	0.8	lithography	1.0
locust bean	0.34	2.7	paper, ice cream, textiles	8.0
psyllium seed	0.35	1.0	cosmetics, textiles, laxatives	3.0
tragacanth	1.50	7.2	pharmaceuticals, salad dressings, dietetic foods	1.6
total		30.2		71.4

[a]Millions of dollars based on average value of sales for 1963.
[b]Millions of pounds in 1963.

Analysis and Identification

The chemistry of the natural gums has been widely studied in the past, and even today the exact structure of most gums has not been accurately determined. Essentially the natural gums are complex polysaccharide polymers combined with certain metallic cations, such as sodium, potassium, calcium, or magnesium. Modern analytical techniques, based on chromatography and infrared spectroscopy, have led to improved isolation and characterization of the basic sugar molecules comprising the total structure, but the exact overall molecular configurations are still not known for most of the natural gums. This has made the separation and identification of gums, especially from mixtures, exceedingly difficult and complicated.

Various chemical and physical tests have been developed to identify pure samples of many gums, and most of these tests are comparatively straightforward. When it is necessary to analyze a mixture of gums in products such as foods, drugs, and cosmetics, it is a much more difficult task and success often depends on the ability to separate and isolate the gums in question. Numerous tests have been devised for the separation and identification of gums by means of various reagents (4). Well-described procedures consist of examining the behavior of gums in water, and their color reactions and types of precipitate produced on addition of standard reagents (5). Final identification of gums is usually accomplished by a combination of methods ranging from simple solubility tests to sophisticated spectrographic analyses.

Thus, gum arabic dissolves readily in cold water to give a tacky, but not very viscous solution, while tragacanth, karaya, or ghatti does not dissolve completely and gives a much more viscous solution than gum arabic of equal concentration (5).

The physical appearance and rate of settling of precipitates formed by treatment of aqueous gum solutions with alcohol may be a preliminary screening test for the identification of gums. For example, in the identification of commercial gums in food products, locust bean gum gives a stringy, opaque, nonadherent precipitate, while gum tragacanth yields a long stringy, adherent precipitate. Karaya forms fine filamentous, nonadherent particles, while agar produces a heavy flocculent precipitate which settles rapidly (6).

In general it is useful to take advantage of specific reactions of the isolated gum with various reagents or dyes. A scheme for the preliminary identification and differentiation of gums by precipitation reactions has been developed (7), and it can be used in conjunction with coagulation reactions by electrolytes (8) and with specific dye reactions (9) to establish a tentative identity for a gum, subject to confirmation by known tests. In addition, methods have been developed for certain gums, such as pectin and carrageenan (10,11), in which specific interest has been shown at various times.

The modern technique of infrared spectrographic analysis is useful in distinguishing the various component sugars and glycosidic linkages (12–14). Sophisticated techniques for obtaining infrared spectra of water-soluble gums have also been developed and standard reference spectra of the common gums have been determined experimentally for the identification of gums (15–17).

With the help of the more recent technique of differential thermal analysis, which has long been used for classification of inorganic compounds, it is possible to differentiate between carbohydrate polymers having different glycosidic linkages. It has been shown that the decomposition of certain simple sugars and polysaccharides is

dependent to some extent on the nature of the glycosidic bonds, which may be identified (18–20).

X-ray crystallographic examination has been used effectively for carbohydrate polymers with a high degree of linearity, such as guar gum, but it is of limited value for the irregularly branched gums (21).

Final gum confirmation can be obtained by hydrolysis of the gum and identification of the sugars separated by paper chromatography. The rate and possibly the order in which the particular sugars are liberated should also be ascertained since certain gums may be differentiated by their stability toward acidic reagents (22,23). Microscopic techniques may confirm the identification of gums (24).

Individual Gums

Gum arabic, or gum acacia, is the oldest and best known of the natural gums and has a venerable history which dates back to ancient Egypt where it was used as an adhesive for mineral pigments in paints. Due to its widespread use over the centuries, it has also many locally descriptive names, such as gum Senegal, Turkey gum, Kordofan gum, Talha gum, and many others.

Gum arabic is the dried exudate obtained from the various species of trees of the genus *Acacia* of the *Leguminosae* family. About 500 species of *Acacia* are found in various tropical and semitropical areas of the world; the most important producing areas are the Republic of the Sudan and some of the countries in West Africa. Poorer-quality gums are also available from acacia trees found in Australia, South Africa, and India. World production of gum arabic is difficult to estimate since such statistics are not readily available, but figures for the United States indicate annual imports of about 20,000,000 lb.

The acacia trees produce gum arabic only under adverse conditions, such as lack of moisture, poor nutrition, or excessively hot weather. The gum is formed in breaks or wounds in the tree bark, which are sometimes caused deliberately, and is exuded in the form of "tear drops." After being collected by hand by natives, the crude exudates are transported to central collecting stations where they are graded by hand sorting and exported to various parts of the world for processing and grinding to meet various specifications.

The exact structure of gum arabic is not known, but it exists in nature as a neutral or slightly acidic salt of a complex polysaccharide containing calcium, magnesium, and potassium salts with a molecular weight of about 240,000–300,000. A proposed tentative structure for gum arabic suggests a main chain of D-galactopyranose units linked $C_1 \rightarrow C_3$, with branched chains on C_6 consisting of D-galactopyranose, D-glucuronic acid, and L-arabofuranose. Some additional side branches of L-rhamnopyranose are also present on the D-galactopyranose side chains (25).

Among hydrocolloids, gum arabic is unique because of its very high solubility in water. It can form solutions of up to 50% concentration whereas most gums are limited to solubilities of less than 5%. This property makes it very useful in applications where low viscosities are required.

Gum arabic solutions are slightly acidic with a pH of 4.5–5.5. The maximum viscosity of the gum solution is at pH 6–7 and is lowered by the addition of electrolytes, protracted heating, or long exposure to acid conditions which may cause partial hydrolysis.

Gum arabic is insoluble in alcohol and other organic solvents. It is slightly soluble in aqueous alcohol, with the solubility decreasing as the proportion of alcohol to water increases up to about 60% alcohol, at which level it is practically insoluble.

The uses of gum arabic are wide and varied. In the food industry, it has achieved great importance in the manufacture of spray-dried "fixed" flavors. These stable, powdered flavors are widely used in packaged dry-mix products, such as puddings, desserts, cake mixes, and similar products, where flavor stability and long shelf life are important (26). It has been used as a base for gum drops, pastilles, and other confectionery products. In bakery products, gum arabic has been used in cake glazes and icings (27); in beverages it performs well as a flavor emulsifier and as a foam stabilizer.

Gum arabic has been a staple in the pharmaceutical industry for many years and the preparation of acacia syrups and specifications for gum arabic are given in the *U.S. Pharmacopeia* (28). These syrups are used in pharmaceutical preparations to aid in the suspension of insoluble drugs and also, conversely, for preventing the precipitation of heavy metals from their solutions through the formation of colloidal suspensions. It is also an excellent excipient in the manufacture of pills and tablets.

Gum arabic finds limited applications in the cosmetic industry as an emulsion stabilizer for lotions and protective creams. In face powders and rouges it is effective as a pigment binder.

As an adhesive medium, gum arabic has found specialized use in glues, transparent cements, library pastes, mounting pastes, and special binding cements for iron castings. In traditional paint and pigment formulations, gum arabic has been effective as a protective colloid and suspending agent, and more recently has been employed in the preparation of vinyl resin emulsions for water-based paints (29). Its protective colloid properties are extremely useful in the formulation of various types of inks, such as government record ink, soluble textile inks, water-color inks, quick drying inks, indelible fabric marking inks, pigmented inks, typographic and hectographic inks, transfer inks, gloss-finish inks, electrically conductive ink, and wood-grain inks. In lithography, gum arabic is used widely in plating and etching solutions, because of its high solubility. It is a sizing and finishing agent in the textile industry and is also used in textile printing and waterproofing formulations. Miscellaneous applications of gum arabic include its use as a corrosion inhibitor in storage batteries; as a plating aid for immersion plating of copper on aluminum; as an emulsifier for drilling fluids; as a glaze binder in ceramics; as a binder for insecticides; and as a nonglare coating for automobile windshields. Derivatives of gum arabic, such as the dicarboxylic acid esters, serve as overcoating and backing layers for photographic materials (30).

Gum tragacanth is the plant exudate of the genus *Astragalus* of the *Leguminosae* family. The name "tragacanth" is derived from the Greek *tragos* (goat) and *akantha* (horn) and probably refers to the curved shape of the ribbons of the best grade of commercial gum. Gum tragacanth is recognized officially in the *U.S. Pharmacopeia* and defined as "the dried gummy exudation from *Astragalus gummifer* Labillardière, or other Asiatic species of *Astralagus* (fam. *Leguminosae*)" (31).

The various species of *Astralagus* are all small, low, bushy shrubs that are found in various sections of Asia Minor and the arid, mountainous regions of Iran, Syria, and Turkey. Iran is the best commercial source and produces the highest-quality gum.

The gum exudes spontaneously from breaks or wounds in the bark of the shrubs and is collected by hand. It is then taken to collection centers where it is sorted into

various grades of ribbon gum (Maftuli) and flake gum (Kharmoni), and exported to various parts of the world. Tragacanth is also known as bassora gum, hog gum, goat's horn, leaf gum, or Syrian gum (32).

As with many other natural gums, the exact chemical structure of tragacanth is still unknown. Most investigators believe that tragacanth is composed of two major components—a major, water-swellable component, known as "bassorin," comprising about 60–70%, and a minor, water-soluble constituent called "tragacanthin." In addition, small amounts of cellulose, starch, and protein are present.

Bassorin appears to be a complex structure of polymethoxylated acids, which probably yields tragacanthin on demethoxylation. Tragacanthin has a ring structure composed of three molecules of glucuronic acid and one molecule of arabinose, with a side chain of two molecules of arabinose. Associated cations found are mainly calcium, magnesium, and potassium. The polysaccharide has a molecular weight of about 310,000.

Gum tragacanth swells in cold water to give highly viscous colloidal solutions or sols which act as protective colloids, and stabilizing and emulsifying agents. It is insoluble in alcohol and other organic solvents.

Solutions of gum tragacanth have extremely high viscosities, possibly the highest of the natural hydrocolloids. These solutions are acidic with a pH of 5.1–5.9 depending on the grade. Maximum viscosity is usually at a pH of 8, dropping sharply on either side (33).

Gum tragacanth is compatible with other plant hydrocolloids as well as proteins and carbohydrates. It is more resistant to acid hydrolysis than most hydrocolloids and is therefore more stable under acidic conditions. Viscosity reduction does occur in the presence of divalent and trivalent cations; tragacanth sols are also incompatible with bismuth salts as evidenced by the formation of gels.

Gum tragacanth finds use in many applications, but is particularly effective as a suspending agent, since it has a very long shelf life and high resistance to acid hydrolysis. It is used widely in pharmaceutical emulsions, jellies, and salves, as well as dental preparations. It has excellent emollient properties which impart a smooth feeling to the skin and it forms a protective coating. Therefore it is used in the cosmetic industry in facial clays, face creams and lotions, and in hair lotions. In the food industry it finds wide application as a thickening or stabilizing agent for ice cream, candies, syrups, toppings, jellies, sauces, salad dressings, citrus oil emulsions, and diabetic syrups (34). It is a binding agent in cigars, while in the textile industry it is useful as a sizing agent for felt and a thickener for printing pastes. It is a sizing agent for paper and also widely used as an emulsifier in furniture, floor and automobile polishes, and in the preparation of stable insecticide emulsions (35).

Gum karaya is the exudate of trees belonging to the genus *Sterculia*, usually of the species *Sterculia urens*. These trees are found in India and all commercial karaya gum comes from that area. Therefore, karaya gum has also been called India gum, Indian tragacanth, sterculia gum, and gum kadaya.

The producing trees are usually cultivated and the gum production is closely supervised. Before collection, during the April-to-June season, holes about 4-in. deep are drilled into the trunk of the tree. Sap oozes out slowly and builds up lumps of exudate weighing up to 5 lb. The crude lumps are allowed to dry on the tree; then they are collected and broken up into pieces, usually smaller than one in. in diameter. The yield and quality of the gum is dependent upon the weather and improves during

warm, dry weather. The crude gum is then transported to Bombay where it is sorted and graded according to color and foreign matter. The highest grades are white, translucent, and almost free of adhering bark, while the lower grades vary from light-yellow to brown and may contain up to 3% of insoluble impurities. After grading, the gum is bulk packed into burlap bags of 170–180 lb and exported to various parts of the world.

In the United States, gum karaya is usually processed further before being sold for final applications. This treatment usually consists of the physical removal of adhering bark, sand, or dirt, by blowing air through the coarse lumps; then further cleaning, grinding, sizing, and blending is necessary to obtain uniform grades of gum. Powdered gum karaya is usually of a white to light pinkish-gray color.

Structurally, karaya is a complex, partially acetylated polysaccharide with an extremely high molecular weight reported to be in the range of 9,500,000. Hydrolytic studies have shown that its constituents are L-rhamnose, D-galactose, and D-galacturonic acid, in an apparent molecular ratio of 4:6:5 (36). It has an acid number that varies from 13.4 to 22.7, and has the peculiar habit of splitting off free acetic acid. This loss has been approximately correlated with the particle size of the gum. The variation in acid number appears to be influenced both by the source of the sample and its age. Trimethylamine has also been identified in the hydrolysis products in minor amounts. Normally gum karaya contains 12–14% moisture and less than 1% insoluble ash.

Gum karaya does not dissolve completely in water to give a true solution, but swells in cold water to give a colloidal sol. In cold water, concentrations of 3–4% are sufficient to produce heavy, pastelike gels. Upon heating under pressure, thick, brown, syrupy solutions of 20–25% concentrations can be prepared. Karaya will also form viscous sols in alcoholic solutions of up to 60% alcohol.

The normal pH of a 1% gum karaya dispersion is about 4.5–4.7 and it has a characteristic buffering property. If small amounts of alkali are added to raise the pH to 7 or 8, the gum will gradually bring it back to the acid side.

The viscosity of a 1% solution at the normal pH of 4.6 is about 3,300 cP and reaches its maximum at about pH 8.5. In an alkaline solution the characteristic short-bodied karaya solutions become irreversibly transformed into ropy, stringy mucilages; presumably this occurs as a result of deacetylation of the karaya molecule.

Gum karaya is compatible with most other plant hydrocolloids, as well as proteins and carbohydrates. It is incompatible with pyrilamine maleate. Electrolytes and excessive acid cause a drop in the viscosity.

Gum karaya has many uses based primarily upon its cold-water-swelling and suspending properties. A large proportion of the imported karaya is used by the pharmaceutical industry as a bulk laxative because of its extremely high water absorbing and bulking properties (37). It is also very effective as a dental adhesive because of its bland taste and firm adhesive properties. In foods it has been used as a stabilizer in ice cream and to control ice crystal growth in ice pops and sherbets (38). It serves as a binder in sausage meat and bologna, and as a stabilizer in salad dressings, cheese spreads, and meringue toppings. In cosmetics it is effective as an emulsifier in hair wave lotions and hair set concentrates. In some applications, such as in the paper industry, karaya is pretreated with alkali in order to deacetylate the molecule, and thereby make it a more effective binder for pulp in the manufacture of long-fibered,

lightweight papers (39,40). In the textile industry, karaya is modified by cooking under pressure to obtain increased solubility. Solutions of 15–18% karaya solids can then be used as thickening agents for printing dyes as well as for textile sizes. Small amounts of karaya are also used in the manufacture of dry cell batteries and as insecticide emulsifiers.

Gum ghatti is an amorphous, translucent exudate of the *Anogeissus latifolia* tree of the *Combretaceae* family. It is a large tree that is found in the dry, deciduous forests of India and Ceylon. The gum exudate occurs in rounded tears and has a glassy fracture. The color of the exudate varies from very light brown to dark brown with the lighter color yielding a higher and better grade of gum. Gum ghatti is also known as Indian gum.

Since ghatti grows in the same areas as gum karaya, harvesting and grading methods are similar. The gum is formed as a protective sealant when the tree bark is damaged, and is in a soft plastic condition when it is first exuded. These exudates are hand picked by natives on plantations and laid to dry in the sun for several days. It is then transported through mountain "ghats" or passes to Bombay where it is auctioned to exporters. The gum is subsequently hand-sorted and classified into various grades according to color and impurities, and finally packed in 196-lb burlap bags and exported.

After importing, the gum tears are further processed and purified, usually by grinding to a fine-mesh size and removing impurities by sifting, aspiration, and density-table separations. The final powdered material has a gray to reddish-gray color, depending on the grade.

As with many other natural gums, the exact chemical structure of ghatti is not known. It is basically the calcium salt of a polysaccharidic acid, ghattic acid. The purified acid has an equivalent weight between 1340 and 1735. Complete acid hydrolysis of the gum yields the following basic sugar constituents (41): L-arabinose, 5 moles; D-galactose, 3 moles; D-mannose, 1 moles; D-xylose, 0.5 moles; and D-glucuronic acid, 1 mole. The soluble portion of the gum has a molecular weight of 11,860 as determined by osmotic pressure measurements (42).

Only about 90% of gum ghatti is soluble in water and this portion yields a colloidal dispersion with an acidic pH of about 4.5. It is a natural buffer and small amounts of acid or alkali will not effect its viscosity. Higher levels of acid and alkali will overcome this buffering action and cause changes in the viscosity.

Gum ghatti has been utilized in foods and pharmaceuticals as an effective emulsifier for oil and water emulsions. It has been used extensively in oil-well drilling fluids to enhance the properties of viscosity, thixotropy, and low fluid loss. Powdered gum ghatti has been used as a waterproofing constituent in explosives (43). It has been employed as a glaze or varnish in paints and also as a binder in coating compositions. Other applications include as a thickener for textile printing, as a sizing agent for yarn, and as an adhesive for binding cloth to the printing tables. In ceramics it has been used as a binder to enhance the wet strength of clay prior to firing. Related uses have been in adhesives, oil-resistant protective films, paints for concrete, varnishes for artificial leathers, and impregnants for woven fabrics.

Locust bean gum is produced by milling the seeds of the leguminous plant *Ceratonia siliqua* Linn., which is widely cultivated in the Mediterranean area and to a small extent in California. It is also known as carob seed gum, St. John's bread, swine's bread gum gatto, jandagum, or tragasol.

Unlike most gums, locust bean gum is not an extract or exudate, but is essentially a flour produced by milling the kernel endosperms of the tree pod. These brown pods, 8–10 in. long, are produced by the locust tree and usually contain about 10 seeds or kernels. The seed coat or endosperm, which comprises about 30-33% of the seed, is the source of the gum. Production of the gum involves dehusking the tough seed coat and separating the endosperm from the yellow germ, usually by various mechanical rolling and milling operations. The isolated endosperm is then ground into a fine powder and graded according to well-defined standards of color, impurities, viscosity, and related specifications (44).

Structurally, locust bean gum is a neutral galactomannan polymer consisting of a main chain of D-mannose units with a side chain of D-galactose on every fourth or fifth unit. It contains no uronic acids, little or no pentose, and has a reported molecular weight of 310,000.

Locust bean gum is not completely soluble in water due to the presence of small amounts of proteins and impurities. It swells partially in cold water but attains a much greater viscosity when heated to approx 180°F. On cooling, a further increase in viscosity is observed.

Solutions of locust bean gum have comparatively high viscosities and are not appreciably affected by changes in pH. The normal pH of a 1% solution is about 5.3.

Locust bean gum is compatible with other plant gums as well as carbohydrates and proteins. Neutral salts, such as sodium chloride, have little effect on locust bean gum sols, but electrolytes, such as lead acetate, phosphotungstic acid, and tannic acid, precipitate it from aqueous solutions (33).

Dispersions of locust bean gum do not gel, but the addition of borate in neutral or alkaline solutions will gel dispersions to give plastic, ropy, and transparent gels. These gels are cohesive, show no syneresis, and can be reliquefied by the addition of acid, mannitol, or glycerol. Locust bean gum can influence the gelling of other polysaccharides, such as carrageenan and agar. The effect is to make the gels resilient and elastic, rather than brittle and crumbly.

Locust bean gum has found widespread use in the food industry especially as a stabilizer in ice cream mixes where its excellent swelling and water-imbibing properties impart a smooth meltdown and desirable shock resistance to the final ice cream product (45). It is also used as a stabilizer in sauces, salad dressings, and pie fillings. Admixture to soft cheeses increases the yield of curd solids and improves the cheese texture. It is an effective binding and lubricating agent in extruded meats, such as sausages, bologna, and salami. In bakery products the addition of locust bean gum to doughs produces softer, more resilient, and fresher cakes, biscuits, etc. In the pharmaceutical and cosmetic industry, locust bean gum is used as an excipient for tablets, a thickener for toothpaste, and a stabilizer for creams and lotions.

The excellent film-forming properties of locust bean gum have made it a useful sizing and finishing agent for textiles. It is a textile print thickener for various types of dyestuffs. In the paper industry the use of locust bean gum as a beater additive has resulted in improved fiber bonding and thereby increased strength in the finished paper. Locust bean gum, because of its neutral structure, is effective as a drilling-mud additive where resistance to brine and other electrolytes is required. It is a valuable adjunct for use as a binder, thickener, and fixing agent for enamels, steatite masses, porcelain, and other ceramics. It has also been used in the manufacture of photographic papers and gun powder.

Guar gum is found in the seed of the leguminous plant *Cyanopsis tetragonolobus.* This plant, which resembles the soybean plant, is grown extensively in Pakistan and India, and was introduced into the southwestern United States during the early part of this century. The bean pod, about 6 in. long, contains five or six seeds which are considerably smaller than the locust bean seed.

Guar gum is processed in a similar way to locust bean gum. The light-colored seed coat is removed by passing the seeds rapidly through heat and then removing it by a pearling operation. The endosperm, comprising 35–42% of the seed, is then freed from the germ, and ground to a white flour which is the commercial guar gum. Several grades are available depending on color, mesh size, viscosity, and rate of hydration.

Structurally, guar gum is very similar to locust bean gum. It is composed basically of a straight chain of D-mannose with a D-galactose side chain on approximately every other mannose unit. The ratio of mannose to galactose is 2:1 in guar as compared to 4:1 in locust bean gum. Guar gum has a molecular weight on the order of 220,000 (46).

Guar gum swells in cold water to form highly viscous solutions at very low concentrations. The solutions are somewhat cloudy due to the small amount of insoluble fiber and cellulosic material present in the flour. The pH of a typical 1% solution of guar is between 5.5 and 6.1 and it tends to become more acid upon standing. These solutions exhibit a buffering action and are stable over a wide pH range of 4–10.5.

Guar is compatible with other plant gums, such as agar, arabic, karaya, and tragacanth, as well as with starch, gelatin, and other water-soluble proteins. The presence of soluble salts and water-miscible solvents alters the swelling rate of guar sols. The presence of dissolved borate ions particularly inhibits the swelling and lowers the viscosity of guar. Guar solutions can be gelled by the addition of small amounts of borax. The borate ion acts as a crosslinking agent and forms rubbery gels which can be reversed by adjusting the pH to the acid side.

The versatile properties of guar gum have led to its widespread use in the food industry. It is effective as a stabilizer in the manufacture of frozen confections, such as ice cream, icepops, and sherbets. It improves the spreadability and moisture retention characteristics of cream cheese, and cheese spreads and dips. It is used as a binder in processed meats and in canned meat for dog food. It is an excellent thickener for many bakery applications, such as pie fillings, icings, toppings, etc, and is an effective emulsifier and stabilizer for salad dressings and sauces (47).

In pharmaceuticals it is used as a tablet binder and disintegrant; as an appetite depressant; as a laxative; and for treatment of peptic ulcers. In the related cosmetic field, its emulsifying and stabilizing properties are employed in the manufacture of jellies, lotions, creams, suspensions, and emulsions.

It has also found diversified industrial applications, such as a beater additive to improve the strength of paper; as a sizing and finishing agent for textiles; and as a thickener for textile dyestuffs. In the mining industry it has been utilized as a flotation agent and foam stabilizer. In ceramics it is a valuable adjunct for use as a binder, thickener, and fixing agent for enamels, steatite, and porcelain. It is effective as a suspending agent for water-based paints, as well as a thickener for battery electrolytes, printing inks, agricultural sprays, calking materials, and mastics.

Psyllium seed gum is derived from plants of the genus *Plantago;* several species are used as commercial sources. The most common sources are the *Plantago ovata* of India, the *P. indica* of France, and the Spanish *P. psyllium.* Some *P. ovata* is also

grown in the United States on a semicommercial basis. This product is also known as plantain seed or flea seed.

The gum is located in the coating which completely surrounds each seed and can be removed by cracking the seeds. The gum is then extracted from these seed coats or husks by extraction with boiling water followed by mechanical separation from cellulose and other insoluble materials.

Psyllium seed gum consists apparently of mixtures of both neutral polysaccharides and acidic polysaccharides which contain D-galacturonic acid residues. The basic composition varies from species to species; extracts of the *P. ovata* species contain the following sugar components: D-xylose, L-arabinose, L-rhamnose, and D-galacturonic acid (48,49).

Psyllium seed gum hydrates slowly and forms dispersions of relatively high viscosity at concentrations up to 1.0%. At higher concentrations of about 2.0% clear gel-like masses are formed.

Industrial applications of psyllium seed are few; its main use is as a bulk laxative in pharmaceutical preparations. It may also be used as a sclerosing agent for intravenous treatment of varicose veins. Since it has shiny and stringy properties, it can be used in cosmetics as a hair-setting lotion. The gum may also be employed in the sizing of silks, textile printing, and paper manufacture.

Quince seed gum is the product of the common quince tree, *Cydonia vulgaris* or *C. oblonga* of the family *Rosaceae*, subfamily *Pomeae*. The tree has been cultivated since prehistoric times in the temperate regions throughout the world. The chief producing areas are in Iran, which supplies about 75% of the total world production. Other sources are Spain, Portugal, India, South Africa, and Iraq.

To obtain the gum, the quince fruit is allowed to rot or dry in air. The seeds are separated by hand, collected in small lots, and sold to traders or exporters. The seeds are then cleaned and graded before exporting. The value of the seed depends upon the gum content (about 20% by wt), freedom from adulteration, and color of the gum solution. Quince seed is also known as semen cydonia, golden apple seed, or cydonium seed. The gum is present in the tough, whitish coating which covers the seed. The gum is soluble in cold water, but much more soluble in hot water, and gives relatively viscous solutions at concentrations up to 2%.

Chemically, quince seed gum, is considered to be a complex combination of a cellulose component and a readily hydrolyzable polysaccharide. Mild acid hydrolysis of the gum yields cellulose, L-arabinose, and a mixture of aldobiuronic acids. The acids are probably D-xylose combined with monomethylhexuronic acid or with unmethylated hexuronic acid (50,51). Electron photomicrographs of its structure show cellulose microfibrils suspended in an amorphous medium composed of glycuronoglycans (52,53).

Quince seed gum is used primarily by the cosmetic industry as an ingredient in hand lotions, wave-setting preparations, cream mascara, and liquid cleansing milk. In foods, it is a stabilizer in chocolate milk and ice cream (54). Its emulsifying and stabilizing properties have led to use in pharmaceutical proprietaries and it has been suggested for drilling operations in areas where brine may be encountered when drilling for oil (55).

Larch gum (arabinogalactan) is a new water-soluble natural gum obtained by the water extraction of the western larch tree (*Larix occidentalis*) which is indigenous to the northwestern United States. This gum was first described in 1898 and several

unsuccessful attempts were made to develop this material commercially. In the early 1960s a new extraction process was developed at Washington State University under sponsorship of the St. Regis Paper Co. Now a domestically produced gum of uniform quality is commercially available.

Structural studies indicate that arabinogalactan is a complex, highly branched polymer of arabinose and galactose in a 1:6 ratio. Molecular weight studies indicate that it is composed essentially of two fractions of widely disparate degrees of polymerization. One fraction, comprising about one-third the total weight, has an average molecular weight of 16,000, while the other fraction, comprising the bulk of the polymer, has a molecular weight of 100,000 (56). Subsequent studies at Washington State University indicated an overall average molecular weight in the range of 72,000–92,000.

Commercial larch gum is a light tan-colored powder that dissolves readily in hot or cold water to give light amber colored solutions. It is extremely soluble and has very low viscosities so that solutions of over 40% solids can be easily prepared. The pH is about 3.8 over concentrations ranging up to 40%. It shows excellent compatibility with electrolytes and is not susceptible to "salting out." It has good heat stability characteristics and although the viscosity of solutions decreases upon heating, the original value is regained upon cooling. Larch gum exhibits pronounced surfactant properties and has not only the effect of substantially reducing the surface tension of water solutions, but it also has a similar effect on the interfacial tension existing in water and oil mixtures.

Larch gum is nontoxic (57) and has been approved for food use as an emulsifier, stabilizer, binder, or bodying agent for essential oils, nonnutritive sweeteners, flavor bases, dressings, and pudding mixes (58). It has been used as a dispersing agent for mineral filler systems, such as clay carbonate, titanium dioxide, etc. It has found extensive use in the lithography industry and has been considered for use in paper coatings (59).

Other Gums. In addition to these economically important gums, many other natural gums are available, which are not used widely in commerce, but which may be obtained if desired.

Some of these, such as tamarind gum in India, or ti gum in Hawaii, are being investigated and used locally, and may well become important in due time, but at present, these, and many others are not sufficiently important to be treated here. For detailed discussions of these lesser known natural gums, see references 1, 2, and 60.

Bibliography

"Gums and Mucilages" in *ECT* 1st ed., Vol. 7, pp. 329–339, by D. C. Beach, S. B. Penick & Company.

1. F. Smith and R. Montgomery, *The Chemistry of Plant Gums and Mucilages*, Reinhold Publishing Corp., New York, 1959, pp. VII–VIII.
2. R. L. Whistler, *Industrial Gums*, Academic Press, Inc., New York, 1959, pp. 2–6.
3. *Oil, Paint, Drug Reptr.* (Jan. 27, 1964).
4. W. O. Walder, *Food* 18, 86 (1949).
5. M. B. Jacobs and L. Jaffe, *Ind. Eng. Chem. Anal. Ed.* 3, 210 (1931).
6. H. N. Bundesen and M. J. Martinek, *Milk Food Tech.* 17, 79 (1954).
7. M. H. Ewart and R. A. Chapman, *Anal. Chem.* 24, 1460 (1952).
8. H. Deuel and H. Neukom, *Advan. Chem. Ser.* 11, 51 (1954).

9. H. G. Bungenberg de Jong and B. Kok, *Proc. Acad. Sci. Amsterdam (in English)* **43**, 728–731 (1940); *Chem. Abstr.* 36, 6873 (1942).
10. R. M. McCready and E. A. McComb, *Anal. Chem.* 24 (12), 1986 (1952).
11. H. D. Graham, *Food Res.* 25, 720 (1960).
12. S. A. Barker, E. J. Bourne, and D. H. Whiffen in D. Glick, ed., *Methods of Biochemical Analysis*, Vol. 3, Interscience Publishers, Inc., New York, 1956, p. 213.
13. L. P. Kuhn, *Anal. Chem.* 22, 276 (1950).
14. R. L. Whistler and L. R. House, *Anal. Chem.* 25, 1463 (1953).
15. S. H. Newburger, J. H. Jones, and G. R. Clark, *Proc. Sci. Sect. Toilet Goods Assoc.* **18**, 38 (1952).
16. *Ibid.*, 19, 25 (1953).
17. J. A. McNulty, *J. Assoc. Offic. Agr. Chemists* 43 (3), 624 (1960).
18. H. Morita and H. M. Rice, *Anal. Chem.* 27, 336 (1955).
19. H. Morita, *Anal. Chem.* 28, 64 (1956).
20. H. Morita, *J. Am. Chem. Soc.* 78, 1397 (1956).
21. K. J. Palmer and M. J. Ballantyne, *J. Am. Chem. Soc.* 72, 736 (1950).
22. E. L. Hirst and J. K. N. Jones in K. Paech and M. V. Tracey, eds., *Modern Methods of Plant Analysis*, Springer-Verlag, Berlin, 1955, p. 275.
23. R. Grau and A. Schweiger, *Z. Lebensm. Untersuch. Forsch.* 119, 210 (1963).
24. A. T. Czaja, *Z. Lebensm. Untersuch. Forsch.* 117, 499 (1962).
25. M. Glicksman and R. Schachat in reference 2, pp. 232–298.
26. G. Wellner, *Food Eng.* 25 (8), 94 (1953).
27. S. J. Werbin, *Southern Dairy Prod. J.* 53, 38 (1953).
28. *U.S. Pharmacopeia, XVI*, Mack Publishing Co., Easton, Pa., 1960, p. 23.
29. U.S. Pat. 2,508,343 (1950), W. K. Wilson (to Shawinigan Resins Corp.).
30. Brit. Pat. 554, 758 (1943), C. R. Fordyce and J. Emerson (to Kodak Ltd.).
31. Reference 28, p. 767.
32. H. S. Gentry, *Econ. Botany* 11, 40 (1957).
33. G. Meer, Jr. and W. A. Meer, *Am. Perfumer* 77 (2), 34 (1962).
34. M. Woo and C. L. Huyck, *Bull. Natl. Formulary Comm.* 16, 140 (1948).
35. U.S. Pat. 2,528,544 (1950), M. Pijoan.
36. P. S. Rao and R. K. Sharma, *Proc. Indian Acad. Sci.* 45A, 24 (1957); *Chem. Abstr.* 51, 15160 (1957).
37. A. M. Goldstein and E. N. Alter in reference 2, pp. 343–360.
38. N. H. Nash, *Advan. Chem. Ser.* 25, 45 (1960).
39. U.S. Pat. 2,069,766 (1937), T. R. Le Compte (to John A. Manning Paper Co.).
40. U.S. Pat. 2,088,479 (1937), T. R. Le Compte (to John A. Manning Paper Co.).
41. J. Fleischer in reference 2, pp. 311–320.
42. D. Hanna and E. H. Shaw, Jr., *Proc. S. Dakota Acad. Sci.* 21, 78 (1941); *Chem. Abstr.* 36, 3386 (1942).
43. U.S. Pat. 2,680,068 (1954), S. H. Davidson and C. H. Rigby (to Imperial Chemical Industries, Ltd.).
44. M. Glicksman, *Advan. Food Res.* 11, 162 (1962).
45. F. E. Potter and D. H. Williams, *Milk Plant Monthly* 39 (4), 76 (1950).
46. G. Meer, Jr., and W. A. Meer, *Am. Perfumer* 77 (4), 49 (1962).
47. A. M. Goldstein and E. N. Alter in reference 2, pp. 321–342.
48. R. A. Laidlaw and E. G. V. Percival, *J. Chem. Soc.* 1949, 1600.
49. *Ibid.*, 1950, 528.
50. A. G. Renfrew and L. H. Cretcher, *J. Biol. Chem.* 97, 503 (1932).
51. K. Bailey and F. W. Norris, *Biochem. J.* 26, 1609 (1932).
52. K. Muhlethaler, *Makromol. Chem.* 2, 143 (1948).
53. K. Muhlethaler, *Exptl. Cell. Res.* 1, 341 (1950).
54. G. Hadary, *Food Inds.* 15, 76 (1943).
55. G. Tchillingarian and C. M. Beeson, *Petrol. Eng.* 24 (4), 45 (1952); *Chem. Abstr.* 46, 5299 (1952).
56. H. O. Bouveng and B. Lindberg, *Acta Chem. Scand.* 12, 1977 (1958); *Chem. Abstr.* 54, 22386 (1960).

57. M. R. Nazareth, C. E. Kennedy, and V. N. Bhatia, *J. Pharm. Sci.* 50 (7), 560 (1961).
58. *Federal Register*, 30 (No. 121.1174), 2430 (Feb. 25, 1965).
59. *Chem. Week*, Aug. 11, 1962, p. 41.
60. C. L. Mantell, *The Water Soluble Gums*, Reinhold Publishing Corp., New York, 1947.

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GYPSITE. See Calcium compounds, Vol. 4, p. 17.

GYPSUM, $CaSO_4.2H_2O$. See Calcium compounds, Vol. 4, p. 16.

H

H ACID, $H_2N(OH)C_{10}H_4(SO_3H)_2$. See Naphthalene derivatives.

HAFNIUM AND HAFNIUM COMPOUNDS

Hafnium, Hf, atomic number 72, is placed in group IV and period 6 of the periodic table between the rare earth metals and tantalum. Hafnium is in the same subgroup as zirconium with which it is always associated in nature and from which its properties differ only very slightly (except for certain nuclear properties). In fact, the two elements are more similar in properties than any other pair in the whole periodic table. This similarity in chemical behavior is related to the electron configuration of the valence electrons, $4d^2$, $5s^2$, and $5d^2$, $6s^2$ for zirconium and hafnium, respectively; and the similarity in ionic radii of the M^{4+} ions, Zr^{4+}, 0.74 Å, and Hf^{4+}, 0.75 Å (1). The fact that the hafnium ion is almost the same size as the zirconium ion, although the nucleus of the former has thirty-two additional protons, is a consequence of the lanthanide contraction. The principal valence state of hafnium is 4^+. The aqueous chemistry of hafnium compounds is characterized by the high degree of hydrolysis exhibited, the formation of polymeric species, and the multitude of complex ions that can be formed.

The Bohr atomic theory was the basis for postulating that element 72 should be tetravalent rather than trivalent. Following a suggestion by Bohr, Coster and von Hevesy examined the x-ray spectra of several zirconium concentrates and found lines at the positions and with the relative intensities postulated by the Bohr theory. The discovery of hafnium was announced in 1923 (2,3). Hafnium was named after Hafnia, the Latin name for Copenhagen, where Coster and von Hevesy discovered the element.

Hafnium is used as a neutron absorber material, usually in the form of control rods, in nuclear power reactors. Hafnium is also gaining acceptance as a high-temperature strengthening agent in alloys of niobium, tantalum, molybdenum, and tungsten.

Physical Properties. Hafnium is a hard, shiny, ductile metal with a color very similar to that of stainless steel, although the color of hafnium sponge metal is a dull powder gray. Selected physical properties of hafnium are given in Table 1.

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square of the concentration except near the melting point or when M_w is small indicates that, with the latter exceptions, the ratio of useless to total bonds is independent of concentration. Either $G \propto (n_t - n_a)$, as would be expected for rubberlike elasticity, and $n_t \propto c^2$, following a binary association; or $G \propto (n_t - n_a)^2$, which might correspond to a stiff strutted structure, and $n_t \propto c$, a peculiar type of association recently postulated by Doty for polyvinyl chloride solutions.[20] At present it is not possible to distinguish between these two alternatives.

Decrease in rigidity with increasing temperature must be due primarily to decrease in n_t. At the same time, the change is probably enhanced, at least near the melting point, by an increase in n_a, since the contributions to n_a from loose ends remain constant and those in the sol fraction should increase.

The decrease in rigidity with decreasing average molecular weight is clearly not due solely to the increase in loose ends, since in this case the total number of bonds should be constant and either G or $\sqrt{G}$ should be a linear function[19] of $1/M_n$. The total number of bonds evidently varies with average molecular weight, and with molecular weight distribution. Further work will be needed to explain the form of the empirical relationship given in equation (1).

(20) P. Doty, H. Wagner and S. Singer, *J. Phys. Colloid Chem.*, **51**, 32 (1947).

Summary

1. The rigidity of a gelatin gel at a given temperature reaches a constant value more rapidly if the temperature is approached from below than from above.

2. For a sample of slight degradation (M_n = 45,000), the rigidity was closely proportional to the square of the concentration up to 60 g./l. At higher concentrations, it increased somewhat less rapidly; for a sample of higher degradation, somewhat more rapidly, than with the square of the concentration.

3. For all samples and at all concentrations, the rigidity decreased gradually with increasing temperature from 0° to the melting point.

4. The rigidity decreased markedly with increasing degradation, or decreasing average molecular weight.

5. An empirical equation for the dependence of rigidity on temperature and weight average molecular weight is given.

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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION]

Chemical Composition and Properties of Guar Polysaccharides[1,2]

BY EILEEN HEYNE AND ROY L. WHISTLER

Endosperm of the guar seed consists principally of a galactomannan polysaccharide. It is thus analogous to the endosperm of locust beans which are widely used in commerce, and to galactomannans from other sources. Guar, a drouth-resistant legume of the genus *Cyamopsis*, is native to India where it is used for food and feed. The endosperm can be employed industrially in many ways, such as a size for paper and textiles, a dispersing agent, and a thickener. These uses have resulted in the recent growing of the guar plant in commercial quantities in the United States. As yet, however, little information is available with regard to the fundamental composition and structure of the endosperm polysaccharides. This report covers a preliminary investigation of the general composition and properties of the guar endosperm and particularly of the water soluble component which constitutes the major portion of the endosperm.

Experimental

Material.—Guar flour, produced by grinding the endosperm of the decorticated guar seed, was obtained through the kindness of General Mills, Inc. The grayish-white flour contained 0.60% nitrogen, 0.06% phosphorus, 1.06% ash and 1.5% ethanol solubles from twenty-four hours of Soxhlet extraction.

Analytical Methods.—Galactose anhydride content was determined by the following procedure: One gram of polysaccharide material ground to pass a 60-mesh sieve was dissolved in 150 ml. of 5% nitric acid by heating the mixture to 100°. After hydrolysis for three and one-half hours at this temperature, the solution was concentrated on a steam-bath to a volume of 25 ml. and 5.6 ml. of concentrated nitric acid was added to make a solution concentration of 25%. The solution was then oxidized according to the methods proposed by Tollens,[3] Van der Haar,[4] and Wise and Peterson.[5] The weight of the solution was reduced to 20 g. by heating on a steam-bath. Because of the high galactose content of the preparations, it was not found necessary to add the 500 mg. of pure mucic acid recommended by earlier works. The crystallization of mucic acid was allowed to proceed for forty-eight hours at a temperature of 0° ± 0.1° obtained with a large constant temperature bath. The solubility of mucic acid at 0° is 0.0175 g. per 100 ml. The amount of galactose anhydride equivalent to mucic acid was found by multiplying the weight of mucic acid obtained in this procedure by the factor 1.33. This factor was computed from data obtained on the analysis of a mixture of galactose and mannose combined in such proportions as to give the same yield of mucic acid as the guar samples.

(1) Journal Paper No. 319 of the Purdue University Agricultural Experiment Station.

(2) Paper presented before the Division of Sugar Chemistry and Technology at the 111th meeting of the American Chemical Society, Atlantic City, 1947.

(3) B. Tollens, *Ann.*, **227**, 223 (1885); **232**, 187 (1886).

(4) A. W. Van der Haar, *Biochem. Z.*, **81**, 203 (1917).

(5) L. E. Wise and F. C. Peterson, *Ind. Eng. Chem.*, **22**, 862 (1930).

Mannose anhydride content was determined through isolation of mannose phenylhydrazone by Nowotnowna's[6] modification of Schorger's[7] and Tollens'[8] procedure.

Both mannose and galactose are of the D-series as evidenced by the optical rotations of their derivatives. Mannose phenylhydrazone from guar showed the same optical mutarotation as the derivative of D-mannose[9] changing from $[\alpha]^{25}D$ +21 (1 hour) → −3 (33 hours) → +35 (216 hours) (*c*, 1 in pyridine). Melting point and mixed melting point with D-mannose phenylhydrazone were 182.5°. The benzimidazole of galactose prepared from guar hydrolyzate after D-mannose had been removed as the phenylhydrazone agreed with the known rotation of D-galactose benzimidazole[10] $[\alpha]^{25}D$ +43.3 (*c*, 0.5 in 5% citric acid).

Pentosans were determined by isolation of the furfural-phloroglucinol complex.[11]

Viscosities were determined at approximately 0.2% concentrations in 1 *N* sodium hydroxide solution at 25°. Solution of the sample was obtained by shaking it overnight in an atmosphere of nitrogen. Viscosity measurements were made twenty hours after the addition of alkali.

Water Fractionation.—An 0.8% aqueous suspension of guar flour was prepared by sprinkling 4-g. portions of the flour into 500-ml. portions of distilled water stirred in a Waring Blendor and by repeating this procedure until 8 liters were obtained. The viscous dispersion was autoclaved at 15 pounds pressure for three hours (*p*H 5.8) and immediately centrifuged in a supercentrifuge (40,000 r. p. m.) to remove the insoluble component. Longer periods of autoclaving did not appreciably change the ratio of soluble to insoluble fractions. The insoluble component was gradually added to ethanol stirred in a Waring Blendor, filtered, and stirred three further times with fresh portions of ethanol in the Blendor. After the final washing and filtration, the brown flocculent precipitate was dried over calcium chloride in a vacuum desiccator. The residue represented approximately 7.8% of guar flour.

The soluble component was recovered by adding an equal volume of ethanol with rapid stirring to the centrifugate; preferably the ethanol was added to small portions of solution stirred in a Waring Blendor. Precipitated material was filtered and washed four successive times with fresh portions of ethanol in the Blendor. The white fibrous precipitate was dried as described above and represented approximately 86.5% of the guar flour. A small amount of carbohydrate was not recovered by this method of treatment. The isolated material contained 0.15% nitrogen, 0.3% or less pentosan, 35.6% D-galactose anhydride and 63.1% D-mannose anhydride. The absence of glucose was indicated by the fact that after nitric acid oxidation of the hydrolyzate and separation of mucic acid, it was not possible to crystallize potassium acid saccharate from the mother liquor. Neither was it possible to isolate glucose as the benzimidazole from the sugar hydrolyzate. Intrinsic viscosity in 1 *N* sodium hydroxide was 5.57; $[\alpha]^{25}D$ +54.5 (*c*, 1 in 1 *N* sodium hydroxide). No uronic acid was detected by the quantitative method of Whistler, Martin and Harris.[12]

Ethanol Fractionation.—Subfractionation of the water soluble component was accomplished by gradual addition of ethanol to the aqueous centrifugate recovered from the separation of insoluble component. Ethanol was added drop by drop from a separatory funnel to 11 l. of the strongly stirred centrifugate. At the first appearance of precipitate the addition of ethanol was stopped and the mixture was supercentrifuged. The solution was allowed to stand for twenty-four hours to allow any additional precipitate to form. If a precipitate formed, the solution was allowed to stand an additional twenty-four hours. If no precipitate occurred, the solution was stirred and further addition of ethanol was made. In this manner a number of fractions were obtained as shown in Fig. 1.

The first precipitate occurred at an alcohol concentration of 20% by volume and the last at 40%. When the alcohol concentration reached 31%, an additional increase of 2% concentration brought about a slow but continuous precipitation which continued for three days. This precipitate which accumulated from 31 to 33% alcohol concentration represented 58% of the total soluble component. Approximately 93% of the soluble component was precipitated by ethanol. Precipitated fractions were washed through four fresh portions of ethanol and dried as described above.

After separation of the above individual fractions, the solution was concentrated under reduced pressure to 1.5 l. and 0.4 l. of ethanol was added. This procedure precipitated fraction number 15. The centrifugate from the precipitation was concentrated under reduced pressure to 280 ml. and 3 l. of ethanol was added to produce fraction number 16. On concentration of the final centrifugate to dryness on the steam bath, fraction 17 was obtained.

Preparation of Guaran.—Material of uniform chemical composition was separated from the water soluble component by discarding the first 10% of material which precipitated up to an ethanol concentration of 25% and then collecting all material which precipitated up to an ethanol concentration of 40% by volume. While only the first 3–5% of ethanol precipitated material appeared to be different from the central fraction, the first 10% which precipitated was discarded simply as a precaution against possible contamination of the central fraction. This material separating between ethanol concentrations of 25–40% and called guaran contained 34.5% D-galactose anhydride, 63.4% D-mannose anhydride and 0.1% nitrogen. The rotation was $[\alpha]^{25}D$ +53 (*c*, 1 in 1 *N* sodium hydroxide). Guaran is not oxidized by Fehling solution. Addition of small amounts of Fehling solution to aqueous solutions of guaran causes the precipitation of a polysaccharide–copper complex.

Acid Hydrolysis of Guaran.—A 1% water solution of guaran was prepared by dispersing 5.50 g. of guaran of known moisture content in 500 ml. of distilled water and by heating this dispersion in an oil-bath at 100° until solution was accomplished. Twenty-five per cent. by weight sulfuric acid was added to make a 1% acid solution. Hydrolysis was carried out at 100°. Rotation measurements were made on 15-ml. aliquots which were removed from the reaction flask and cooled to 25°. The specific rotation changed from an initial value of about +59 to a final value of +37.

Enzymatic Hydrolysis of Guaran.—A 1% water solution prepared as above was treated with a commercial diastase in the proportion of 0.1 g. of diastase for each 100 ml. of solution. Viscosity measurements taken in an Ostwald–Cannon–Fenske tube indicated a decrease to about one-third the initial value in a period of eighteen hours.

Esterification of Guaran.—Freshly precipitated guaran was freed of ethanol by stirring with glacial acetic acid for fifteen minutes and by filtering off the excess reagents. To the pretreated guaran was added 33 parts pyridine and 33 parts acetic anhydride. The mixture was heated in an oil-bath with stirring for four to five hours at 105°. The acetate was then precipitated by pouring the solution into excess ethanol stirred in a Waring Blendor. The precipitate was filtered and washed in the Blendor four successive times with fresh portions of ethanol. The final product was air dried. It was a white, very fibrous material closely resembling amylose triacetate in appearance.

(6) A. Nowotnowna, *Biochem. J.*, **30**, 2177 (1936).

(7) A. W. Schorger, *Ind. Eng. Chem.*, **9**, 748 (1917).

(8) B. Tollens, *Ber.*, **23**, 2990 (1890).

(9) C. L. Butler and L. H. Cretcher, THIS JOURNAL, **53**, 4358 (1931).

(10) S. Moore and K. P. Link, *J. Biol. Chem.*, **133**, 300 (1940).

(11) N. C. Pervier and R. A. Gortner, *Ind. Eng. Chem.*, **15**, 1167 (1923); B. Tollens and E. Krober, *J. Landw.*, **48**, 355 (1900); **49**, 7 (1901); see also Browne and Zerban, "Sugar Analysis," 3rd ed., p. 904.

(12) R. L. Whistler, A. R. Martin and M. Harris, *Bur. Standards J. Research*, **24**, 13 (1940).

Acetyl content, 44.8%; theory, 44.78%; $[\alpha]^{25}D$ 34° (*c*, 1 in chloroform), m. p. 224–226°.

The acetate could be cast into a film by standard methods.[13] The films were lustrous, clear, strong, and flexible. When placed in water at 95–100°, films containing 20% dibutyl phthalate plasticizer could be elongated 550%. The stretched films were birefringent in polarized light but showed no detectable crystallinity on examination with X-rays. When broken under stress, the elongated films developed many longitudinal cracks.

A 2% solution of guaran triacetate dissolved in chloroform was fractionally precipitated by dropwise addition of ethanol with rapid stirring. Seven fractions were obtained each of which possessed a constant rotation of $[\alpha]^{25}D$ 34° (*c*, 1 in chloroform).

Results and Discussion

Guar flour consists principally of carbohydrate material. There is present only 1.5% fatty material or other substances extractable by ethanol. The low nitrogen value indicates the presence of but 3.5–4.0% protein. Only very small amounts of phosphorus-containing compounds are present. From this principally carbohydrate material it is, therefore, not surprising that a water soluble polysaccharide can be easily separated in 86–7% yield. The polysaccharide contains 35.6% D-galactose anhydride and 63.1% D-mannose anhydride. No ketoses[14,15] or uronic acids have been detected in the polysaccharide material. The absence of uronic acids differentiates the polysaccharide from the great majority of plant gums and mucilages.

Information with regard to the homogeneity of the soluble component is obtained by ethanol fractionation of its aqueous solution and comparison of the various fractions. The per cent. of the material received in each fraction is shown in Fig. 1, and the amount precipitated at different concentrations of ethanol is shown in Fig. 2. Comparison of the various fractions as to mannose anhydride content, specific optical rotation, and intrinsic viscosity are shown in Figs. 3, 4 and 5, respectively. These graphs indicate that the first 2.5% of the soluble component to be precipitated is of slightly different composition and lower viscosity than the main portion of the soluble component whose fractions are similar. The lower mannose anhydride content (40.2%) of the first material precipitated may relate it to the water insoluble component which has a mannose anhydride content of 39.1%. It is possible that some of the insoluble component is solubilized by the autoclave treatment. The graphs also indicate that there is present in the soluble component about 3% of very low molecular weight material which judged by its low mannose anhydride content and negative rotation is quite different from the main polysaccharide fractions.

Collectively, the graphs suggest that the water soluble component of guar endosperm consists principally (90–95%) of a polysaccharide which precipitates in a narrow range of ethanol concentration. Further evidence for the homogeneity of the polysaccharide material is the observation that when its acetate in chloroform solution is fractionated into seven parts by gradual ethanol addition, all fractions are found to possess identical optical rotations. Since the polysaccharide contains 34.5% D-galactose anhydride and 63.4% D-mannose anhydride, it may properly be termed a galactomannan. For convenience in designation, this particular polysaccharide fraction in this and future papers is given the name "guaran."

On heating guaran in acid solution it undergoes hydrolysis and the specific optical rotation changes from a value of about +59 to +37. This change from a positive to a less positive value is indicative of the predominance of α-D-glycosidic links and is in agreement with the view of Lew and Gortner[16] who suggest the presence of alpha linkages in the galactomannan of carob bean endosperm. A further indication of the predominance of alpha link-

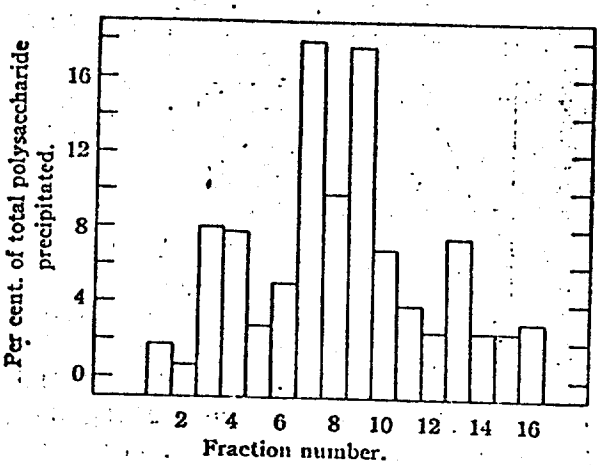

Fig. 1.—Per cent. of guar in each fraction.

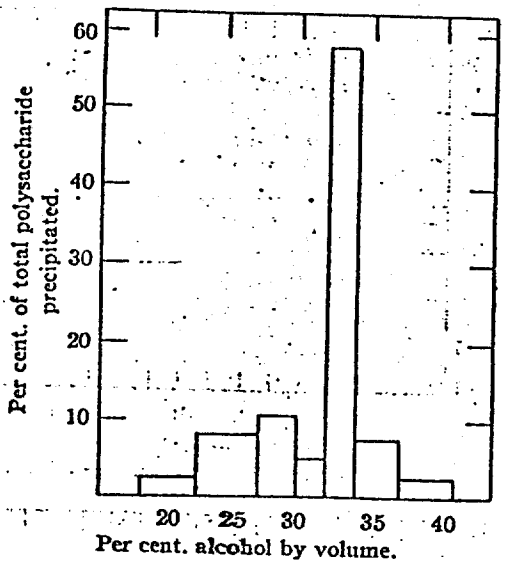

Fig. 2.—Per cent. polysaccharide precipitated at various alcohol concentrations.

(13) R. L. Whistler and G. B. Hilbert, *Ind. Eng. Chem.*, 36, 796 (1944).

(14) B. Th. Seliwanoff, *Ann. Chim. Applicata*, 21, 535 (1931).

(15) R. Ofner, *Chem. Ztg.*, 53, 682 (1929).

(16) B. W. Lew and R. A. Gortner, *Arch. Biochem.*, 1, 325 (1943).

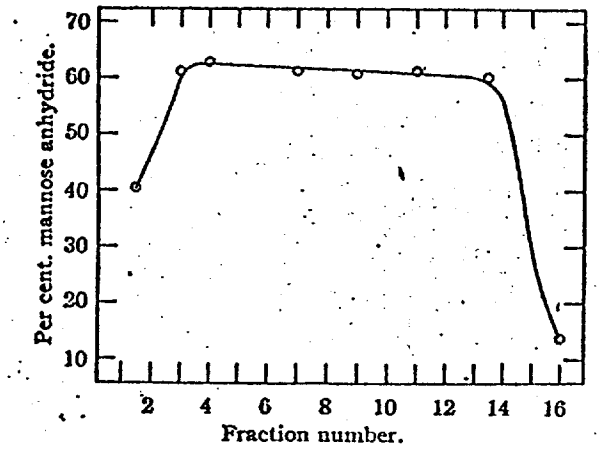

Fig. 3.—Per cent. mannose anhydride in guar fractions.

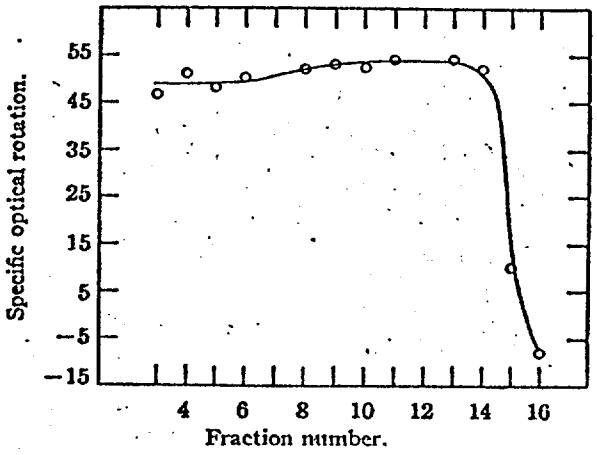

Fig. 4.—Specific optical rotation of guar fractions.

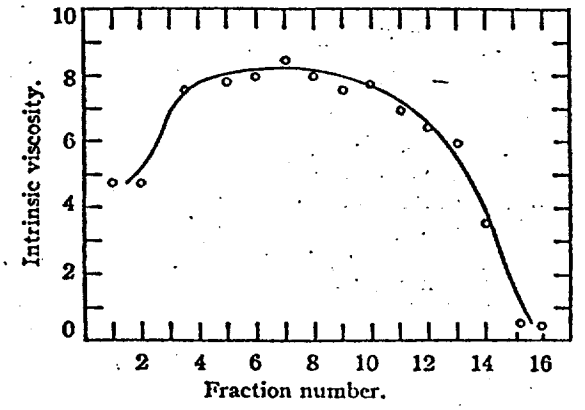

Fig. 5.—Intrinsic viscosity of guar fractions.

ages in guaran is the fact that its solutions are hydrolyzed by diastase, the viscosity decreasing to a value about one-third or less of the original.

Films produced by casting guaran acetates are clear, lustrous, strong, and pliable. Films plasticized with 20% dibutyl phthalate may be easily stretched in water at 100° to elongations of about 550%. During the stretching the film properties change from isotropic to anisotropic. The elongated films when broken under stress tend to shatter in lines parallel to the direction of elongation. Furthermore, a pronounced birefringence is developed during the process of elongation. These occurrences are all indications of the presence of anisodimensional and perhaps linear molecules which are orientated when the film is subjected to plastic flow. However, while the presence of linear molecules is indicated, X-ray analysis of the elongated films failed to give evidence of crystallinity. Films of uniform linear molecules would be expected to produce a fiber pattern or evidence some degree of crystallinity. Failure to obtain this effect may be accounted for by assuming that the guaran chains consist of D-galactose and D-mannose units arranged in random order or that the principal chain may possess branches of very short length. Therefore, although the chains become ordered when the films undergo plastic flow, an orderly three dimensional arrangement of sugar units is not brought about.

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Summary

Guar flour can be readily separated into a water soluble (86–87%) and a water insoluble component. The water soluble component contains only small amounts of nitrogen and phosphorus impurities.

By gradual addition of ethanol to an aqueous solution of the water soluble component, it is separated into 17 fractions. Analyses of the fractions indicate that the third to the twelfth fractions are identical as to composition and represent a galactomannan polysaccharide which contains 34.5% D-galactose anhydride and 63.4% D-mannose anhydride. This polysaccharide is given the name "guaran."

Presence of α-D-glycosidic linkages in guaran is indicated by its rapid acid hydrolysis with accompanying decrease in specific rotation and by its hydrolysis under the action of diastase.

Guaran may be esterified easily to the triacetate which may be cast into a strong, flexible film. On stretching to 550% the film becomes strongly birefringent but yields only an amorphous X-ray pattern. It is assumed that the guaran molecules are linear or highly anisodimensional but have either a random distribution of D-galactose and D-mannose units in the molecular chains or that the chains have very short branches.

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COMPARISON OF METABOLIZABLE ENERGY AND PRODUCTIVE ENERGY DETERMINATIONS WITH GROWING CHICKS

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Accurate data on the energy values of purified nutrients and crude feeding materials are a basic need for quantitative studies in energy nutrition. Presently available information pertaining to avian species is of three kinds. Extensive digestibility data have been summarized by Olsson ('50) and Fraps ('44) for most common feeding materials, and are expressed as digestibility coefficients for protein, fat, crude fiber and nitrogen-free extract. A limited number of directly determined metabolizable energy values are available, and are contained in the reports of Fraps et al. ('40), Olsson ('50), Halnan ('51) and Carpenter and Clegg ('56). Productive energy, an estimate of net energy based on a carcass analysis technique using growing chicks, is a measure that has been employed by Fraps ('46) for the estimation of net energy values of many feedstuffs.

For most purposes, metabolizable energy and net energy are the measures of greatest utility. Digestibility data are subject to the limitations of methods used for chemical or physical separation of feces and urine in mixed excreta, or the difficulties of using surgically altered animals for the separate collection of fecal and urinary wastes. Because of these limitations, the computation of metabolizable energy values from

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[2] These studies were conducted in the Nutrition Laboratories of the Department of Poultry Husbandry.

available digestibility data is not completely satisfactory, although useful information has been obtained in this way by Axelsson and Ericksson ('51) and Titus ('55). Furthermore, some of the samples used in early experiments are not representative of present day feeding materials. Although in theory some measure of net energy would be the most valuable criterion of energy value, the uncertainty of net energy measurements with other species makes it desirable to obtain confirmatory evidence to supplement the data of Fraps for chickens. The purpose of the experiments to be described was to compare metabolizable enery and productive energy determinations using growing chicks in order to establish which of these measures is the most useful for quantitative studies in energy nutrition of the chick and for evaluation of feeding materials.

MATERIALS AND METHODS

All of the experiments to be described were conducted using diet E9, a semi-purified reference diet the composition of which is shown in table 1. The diet was formulated to be adequate in all recognized nutrient requirements of the chick, and liberally fortified with respect to unidentified nutrients as supplied by fish meal, fish solubles, brewers' dried yeast and dried whey. Male cross-bred (RIR $\times$ BPR) chicks were used in all experiments. They were reared to 9 or 10 days of age on diet E9 and were allotted to groups of 10 chicks at this time by a procedure similar to that of McKittrick ('47) to equalize both body weight and rate of gain among experimental groups. The length of the experimental period was 14 days following the formation of the groups.

Productive energy was determined by a method similar to that of Fraps ('46) excepting that each treatment was applied to a group of 10 chicks rather than to individual chicks. The Fraps procedure is based on the difference in energy gain produced by feeding a given diet at two planes of food intake, normally 100% and 50% of ad libitum, using the following expression to compute productive energy: $WM + G = FX$.

in which W is the average chick weight by periods computed from the weights at the beginning (W_1) and after 7 and 14 days of experiment (W_2, W_3) by the expression $\frac{W_1 + 2W_2 + W_3}{4}$; M is the maintenance requirement per unit weight; G is the energy gain computed from carcass compostion of the experimental lots and a lot sacrificed at the beginning of the experimental period; F is the food intake; and X equals productive energy value of the diet per unit weight. Observations on each

TABLE 1

Composition of diet E9

COMPONENT	PER 100 GM	COMPONENT	PER 100 GM
	gm		*gm*
Glucose [1]	44.1	Fish solubles (dry basis)	1.0
Ground wheat	9.0	Hydrogenated vegetable fat [2]	2.5
Soybean oil meal (44% protein)	17.5	Ground limestone	2.0
Crude casein	10.5	Dicalcium phosphate	1.0
Gelatin	2.5	Salt (iodized)	0.5
Fish meal (menhaden)	4.0	Mineral mixture [3]	0.4
Dried brewers' yeast	2.5	Vitamin mixture [3]	0.5
Dried whey	2.0		100.0

[1] Cerelose.

[2] Hydora, Lever Bros.

[3] The mineral and vitamin mixtures supply, in milligrams per 100 gm of diet: 220 K_2HPO_4, 120 $MgSO_4$, 30 $MnSO_4$, 30 $FeSO_4 \cdot 7H_2O$, 0.8 $CuSO_4 \cdot 5H_2O$, 0.3 thiamine, 0.4 riboflavin, 1.0 calcium pantothenate, 0.5 pyridoxine, 2.6 niacin, 0.07 folacin, 0.09 menadione, 0.01 biotin, 0.001 vitamin B_{12}, 130 choline chloride, 1000 USP units vitamin A, 100 I.C. units vitamin D_3, 2.2 mg α-tocopheryl acetate.

lot supplied data for W, G and F, leaving M and X as unknowns. Simultaneous equations for the two lots of chicks fed at different planes of intake permit solving the expressions for X, on the assumption that maintenance requirement per unit weight is essentially constant over the weight range concerned, and that productive energy value of the diet is the same at the two planes of intake.

As conducted in this laboratory, the determinations included analysis of initial body composition using a representative group of 10 chicks sacrificed at the beginning of the experi-

mental period. The small differences in average initial weight among the experimental groups were compensated for in estimating their initial composition by assuming that absolute composition and average weight were proportional over this narrow range. Final composition was determined by sacrificing each experimental group at the end of the 14-day experimental period. The chicks were killed by dislocating the neck without blood loss, the contents of the digestive tract were removed and the carcasses frozen. After passing them through a meat grinder, the carcasses for each lot were dried from the frozen state in a large freeze-drying apparatus. The dried material was equilibrated with atmospheric moisture and ground in a Wiley mill, after which it was thoroughly mixed, sampled and analyzed for moisture, fat (ether extract) and protein ($N \times 6.25$). Successive weighings at each step of the preparation procedure were made so that mechanical losses were accounted for and the absolute amounts of fat, protein and energy were determined for each lot. Energy gains were computed from fat gain at 9.35 Cal. per gram, and protein gain at 5.66 Cal. per gram, as described by Fraps and also by other procedures as discussed later.

Metabolizable energy values were determined by using a composite of mixed excreta from each lot collected on the last three consecutive days of the experimental period. The excreta were frozen after each collection and pooled before drying. Chromic oxide at a level of 0.2% of the diet was used as an index substance in order to eliminate the need for quantitative collections of excreta and quantitative measurement of feed intake (Schürch, Lloyd and Crampton, '50; Dansky and Hill, '52). Analyses were made on feed and excreta as follows:

Moisture: by air oven or vacuum oven.

Nitrogen: by macro-Kjeldahl procedure.

Combustible energy: determined in a Parr adiabatic oxygen bomb calorimeter.

Chromic oxide: determined spectrophotometrically following wet ashing according to the following procedure: a sample

containing approximately 10 mg of chromic oxide is transferred to a 100 ml Kjeldahl flask calibrated at 110 ml. Addition of 10 ml of concentrated nitric acid and allowing the mixture to stand overnight makes subsequent digestion easier, but this step is not necessary; analyses in the experiments reported in this paper were made without benefit of pre-digestion, but more recent work in this laboratory has generally employed it. Where nitric acid is used, the mixture is heated over a micro-burner to dryness, and 15 ml of digestion mixture added. The digestion mixture is prepared by dissolving 10 gm of sodium molybdate in 150 ml of distilled water and slowly adding 150 ml of concentrated sulfuric acid; after cooling in an ice bath, 200 ml of 70% perchloric acid are added with stirring. Digestion of the sample is continued over the open flame of a micro-burner until the green color changes to yellow, orange or red, depending on chromium concentration. The flask is immediately chilled in cold water and diluted with approximately 90 ml of distilled water. After cooling, the mixture is brought to volume (110 ml) and allowed to stand overnight to precipitate inorganic matter. Optical density is determined at 430 mμ in a Beckman DU spectrophotometer. Chromic oxide content is computed from a reference curve covering the range 0 to 150 μg of chromic oxide equivalent per milliliter prepared by aliquot dilutions from digestion of 50 mg of pure chromic oxide as outlined above. In our laboratory the linear reference curve has the form $X = 0.3454\ Y$ where X = milligrams chromic oxide equivalent per milliliter, and Y = optical density.

Metabolizable energy was computed as follows from the analytical data, all expressed on a dry matter basis:

E_{diet} = Calories combustible energy per gram of diet dry matter (determined directly by bomb calorimeter)

$E_{excreta}$ = Calories combustible energy in excreta per gram of diet dry matter
$= \text{Calories per gram excreta} \times \frac{Cr_2O_3 \text{ per gram diet}}{Cr_2O_3 \text{ per gram excreta}}$

N = Nitrogen retention per gram of diet dry matter
$= \text{N per gram diet} - \text{N per gram excreta} \times \frac{Cr_2O_3 \text{ per gram diet}}{Cr_2O_3 \text{ per gram excreta}}$

Metabolizable energy per gram diet dry matter $= E_{diet} - E_{excreta} - 8.22\ N$

In the computations summarized above, correction for positive nitrogen balance was made in order to convert all data to a basis of nitrogen equilibrium for comparative purposes. It was assumed that protein tissue if oxidized for energy purposes would yield uric acid as the sole excretory product, and the value 8.22 used above is the combustible energy value of uric acid per gram of nitrogen. This assumption is not strictly correct because normal chicken urine contains only 60 to 80% of nitrogen as uric acid (Katayama, '24; Coulson and Hughes, '30). However, the error in this assumption is probably not great since calculation of the energy value of chicken urine per gram of nitrogen from the data of Coulson and Hughes ('30) yields a value of 8.7 Cal. per gram of urinary nitrogen; furthermore, the assumption that oxidation of body tissue would yield the same pattern of nitrogen excretion products as normally found in the urine of chickens appears to be no better than the assumption that all the nitrogen is excreted as uric acid. In any case, the range of choice is small and the simplest assumption appears preferable.

EXPERIMENTAL

The first two experiments were conducted to determine the effect of plane of food intake on metabolizable energy and productive energy value of the reference diet. This was considered desirable because no such studies in relation to metabolizable energy determinations with chickens are known to the authors, and the effect of plane of nutrition on productive energy estimations had not been investigated by Fraps. It appeared possible that there may be a limiting level of food intake below which accurate estimation of energy value cannot be made because of physiological disturbances. A single lot of 10 chicks was used for each treatment in each of these experiments. In the first experiment, levels of food intake ranging from 100% to 50% of ad libitum by 10% steps were used; in the second experiment, the 90% ad libitum group

was omitted and two additional groups were fed 40% and 30% of ad libitum consumption respectively.

The results of these experiments are presented in tables 2 and 3. Productive energy was calculated by simultaneous equations as described above, pairing the data for each restricted lot with the ad libitum lot of the respective experiment. The productive energy values showed a wide range of variation both between levels of food restriction and within the same treatment in replicate experiments. It was evident that restriction of food intake to 90% of ad libitum did not produce a sufficiently large difference in energy gain and food intake to permit accurate estimation of productive energy. At the other levels of restriction, there was no evident relationship between plane of intake and productive energy value. On the basis of these results, it was concluded that any plane of restriction less than 80% of ad libitum would be sufficient to produce accurately measurable differences in energy gain and feed intake as compared to ad libitum feeding.

In contrast to the highly variable results obtained in the estimation of productive energy, the metabolizable energy value of diet E9 was notably constant within each replicate experiment. There was no apparent relationship between plane of intake and the metabolizable energy value per unit weight of diet. The difference between the average values in the two replicate experiments, approximating 4% of the mean value, was probably due to small differences in the composition of the diet in the two experiments because the diet was formulated on an air-dry rather than dry-matter basis.

On the basis of these findings, 6 additional experiments were conducted to determine the precision of measurement of the productive energy and metabolizable energy values for diet E9. The two planes of food intake chosen were 100% and 60% of ad libitum. The data from these experiments and the corresponding data from the first two are summarized in

TABLE 2

Effect of plane of feed intake on determination of metabolizable energy and productive energy

(Experiment 1)[1]

PERCENTAGE OF AD LIBITUM FEED INTAKE	AVERAGE WEIGHT		AVERAGE GAINS			AVERAGE FEED CONSUMED[2]	ENERGY VALUE OF DIET[2]	
	Initial	Final	Protein	Fat	Energy		Productive	Metabolizable
	gm	*gm*	*gm*	*gm*	*Cal.*	*gm*	*Cal./gm*	*Cal./gm*
100	95.8	300.1	37.0	20.6	402.0	313	—	3.30
90	94.9	282.5	30.9	14.1	306.7	282	4.76	3.25
80	96.7	263.7	28.2	12.0	271.5	251	2.79	3.23
70	94.9	247.7	22.2	7.2	193.0	222	3.24	3.23
60	95.9	216.1	20.8	3.5	150.8	189	2.85	3.26
50	93.1	186.9	16.8	1.0	104.6	158	2.84	3.25
						Average:	3.30	3.25

[1] Experimental period from 9 to 23 days of age. Lot sacrificed at 9 days to provide initial data had following composition: average weight 97.5 gm, protein 15.1 gm, fat 7.3 gm.

[2] Expressed on dry matter basis.

TABLE 3

Effect of plane of feed intake on determination of metabolizable energy and productive energy

(Experiment 2)[1]

PERCENTAGE OF AD LIBITUM FEED INTAKE	AVERAGE WEIGHT		AVERAGE GAINS			AVERAGE FEED CONSUMED[2]	ENERGY VALUE OF DIET[2]	
	Initial	Final	Protein	Fat	Energy		Productive	Metabolizable
	gm	*gm*	*gm*	*gm*	*Cal.*	*gm*	*Cal./gm*	*Cal./gm*
100	105.8	337.4	41.5	20.9	430.2	343	—	3.38
80	106.4	284.4	32.5	15.2	326.4	270	1.61	3.42
70	102.5	248.4	27.1	8.3	231.0	237	3.02	3.38
60	103.7	234.2	25.5	6.5	205.2	203	2.06	3.39
50	105.4	204.8	20.4	1.9	133.2	170	2.36	3.39
40	102.3	176.1	15.4	1.8	70.6	136	2.32	3.47
30	103.2	142.1	10.4	1.2	19.8	102	2.46	3.14
						Average	2.34	3.19

[illegible]

(Experiment 2)[1]

PERCENTAGE OF AD LIBITUM FEED INTAKE	AVERAGE WEIGHT		AVERAGE GAINS			AVERAGE FEED CONSUMED [2]	ENERGY VALUE OF DIET	
	Initial	Final	Protein	Fat	Energy		Productive	Metabolizable
	gm	gm	gm	gm	Cal.	gm	Cal./gm	Cal./gm
100	105.8	337.4	41.5	20.9	430.2	343	—	3.38
80	106.4	284.4	32.5	15.2	326.4	270	1.61	3.42
70	102.5	248.4	27.1	8.3	231.0	237	3.02	3.38
60	103.7	234.2	25.5	6.5	205.2	203	2.06	3.39
50	105.4	204.8	20.4	1.9	133.2	170	2.36	3.39
40	102.3	176.1	15.4	— 1.8	70.6	136	2.52	3.37
30	105.2	142.1	10.4	— 4.2	19.8	102	2.46	3.39
						Average	2.24	3.39

[illegible]

TABLE 4

Summary of eight replicate determinations of metabolizable energy and productive energy of diet E 9

EXPERIMENT	AVERAGE WEIGHT		AVERAGE GAINS			AVERAGE FEED CONSUMED [1]	ENERGY VALUE OF DIET	
	Initial	Final	Protein	Fat	Energy		Metabolizable	Productive
	gm	gm	gm	gm	Cal.	gm	Cal./gm	Cal./gm
1	95.8	300.1	37.0	20.6	402.0	313	3.30	
	95.9	216.1	20.8	3.5	150.8	189	3.26	2.85
2	105.8	337.4	41.5	20.9	430.2	343	3.38	
	103.7	234.2	25.5	6.5	205.2	203	3.39	2.06
3	123.2	346.4	41.7	22.2	443.5	338	3.33	
	125.2	247.1	24.6	7.1	205.4	203	3.34	2.33
4	104.3	322.7	40.2	21.6	429.9	331	3.38	
	109.5	227.7	24.0	5.1	183.1	200	3.37	2.44
5	116.5	329.5	41.1	20.1	421.1	337	3.29	
	117.5	248.3	24.6	6.4	199.2	209	3.40	2.26
6	115.3	359.9	44.0	25.7	489.2	378	3.29	
	113.9	226.8	23.8	3.2	164.5	220	3.36	3.31
7	97.1	290.7	35.0	18.2	368.3	305	3.31	
	100.3	193.7	19.0	3.6	141.1	179	3.38	2.58
8	96.4	310.9	38.1	20.8	410.2	331	3.32	
	98.2	214.3	22.3	5.8	180.2	193	3.40	2.13
						Average ± σ	3.34 ± 0.045	2.49 ± 0.42

[1] The two lots in each experiment were fed ad libitum and 60% of ad libitum respectively.

table 4 and show that the productive energy ranged from 2.06 to 3.31 Cal. per gram of dry matter with a mean of 2.49 ± 0.42 (standard deviation). In marked contrast to the variability of replicate estimations of productive energy, the metabolizable energy of diet E9 was highly consistent in replicate experiments. The 8 estimates based on ad libitum

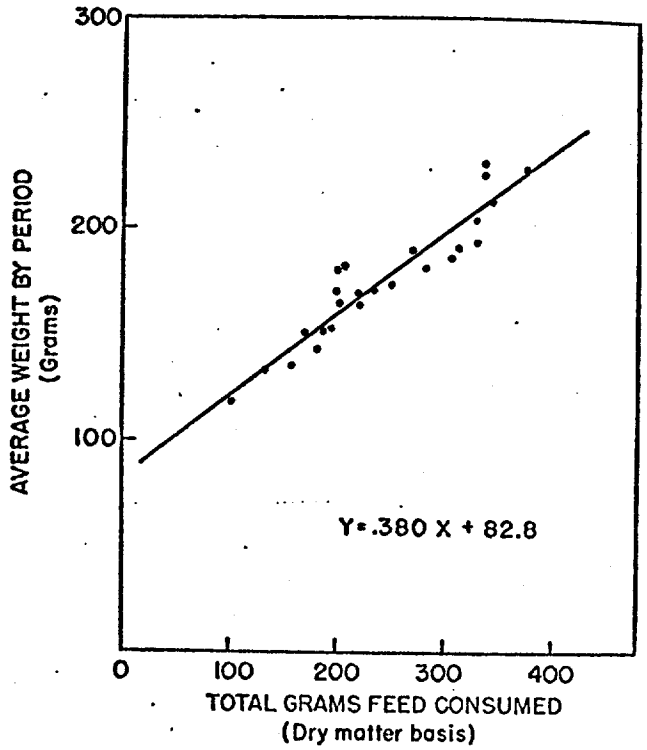

Fig. 1 Regression of average weight by period (Y) on total feed consumed (X) for determining productive energy of diet E9.

feeding averaged 3.32 Cal. per gram, while at restricted intake they averaged 3.36 Cal. per gram. The difference between the values obtained at the two planes of intake was not significant. The over-all mean was 3.34 ± 0.045 Cal. per gram.

To obtain a more valid estimate of the productive energy value of diet E9 than a simple mean of replicate determina-

tions, use was made of a regression analysis technique. The data from all of the experiments presented in tables 2, 3 and 4 were used to compute the regressions of energy gain on total feed consumption and of average weight during the experiment on total feed consumption. The data and the computed

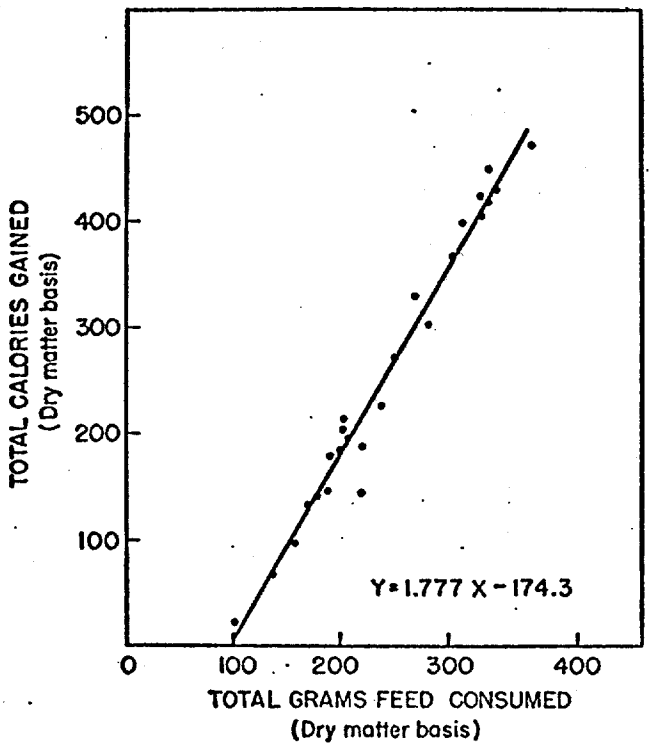

Fig. 2 Regression of total Calories gained (Y) on total feed consumed (X) for determining productive energy of diet E9.

regression lines are shown graphically in figures 1 and 2 together with the linear equations derived for the indicated relationships. By selecting two arbitrary rates of feed consumption, mean values for energy gain and average weight were derived from these regressions for each plane of intake and inserted into the equations previously described for computation of productive energy. This procedure yielded value

of 2.58 Cal. of productive energy per gram of dry matter in diet E9.

DISCUSSION

From the data of these experiments it is clearly evident that metabolizable energy is a highly precise measure of food energy for the chick, and that the estimation of productive energy is associated with a high degree of variation. It is perhaps not surprising that this should be a characteristic of productive energy, since estimations of net energy with other species have also been highly variable.

It is significant to note that the productive energy value obtained for the reference diet in this work was approximately 75% of its metabolizable energy. The large difference between the two measures of energy value, attributable presumably to specific dynamic action (SDA), is considerably greater than would be expected from work with other animals (Swift and French, '54). Whether it represents a distinctively high SDA characteristic of the growing chick or a bias in the method for determining productive energy cannot be deduced from the present data. It should be considered, however, whether two basic assumptions on which the Fraps method is based may be responsible, at least in part. It is assumed that the productive energy value per unit weight of diet is unaffected by plane of food intake. From work with other species, it is considered probable that net energy value is affected by plane of nutrition; the absence of any consistent relation between rate of restricted food intake and the productive energy of the diet in the present work does not necessarily conflict with this view, since the effect of restriction may already have been maximal at a reduction of 20 to 30% below ad libitum consumption.

A further assumption in the Fraps procedure is that maintenance requirement per unit body weight is constant over the range of body weights concerned. It is of course well established that basal metabolic rate and total maintenance

requirement are exponential functions of body weight rather than linear ones. The effect of using a body weight term to the 0.7 power rather than average weight by periods in the simultaneous equations for computing productive energy is shown in table 5, which gives the arithmetic mean and standard deviation for calculations based on the data from the 8

TABLE 5

The effect of using an exponential function of body weight and different energy equivalents for tissue protein in the computation of productive energy of diet E 9

EXPRESSION OF BODY WEIGHT	PRODUCTIVE ENERGY PER GRAM OF DIET E 9	
	Assigning tissue protein 5.66 Cal. per gm	Assigning tissue protein 4.34 Cal. per gm
	Cal./gm	*Cal./gm*
Average weight by periods $\left(\frac{W_1 + 2W_2 + W_3}{4}\right)$	2.49 ± 0.42 (17) [1]	2.32 ± 0.40 (17)
Exponential average weight by periods $\left(\frac{W_1 + 2W_2 + W_3}{4}\right)^{0.7}$	2.18 ± 0.28 (13)	2.01 ± 0.27 (14)
Average of initial and final weights $\left(\frac{W_1 + W_3}{2}\right)$	2.56 ± 0.52 (20)	2.38 ± 0.50 (21)
Exponential average of initial and final weights $\left(\frac{W_1 + W_3}{2}\right)^{0.7}$	2.21 ± 0.31 (14)	2.04 ± 0.31 (15)

[1] Mean ± standard deviation (coefficient of variation, $\frac{100\,\sigma}{M}$).

replicate determinations described in table 4. This change in computation has the effect of reducing the productive energy estimate, and therefore increasing the difference between it and metabolizable energy. If the large SDA effect is an artifact, it is evidently not due to the use of a linear rather than exponential function of body size in estimating maintenance cost. Also shown in table 5 is the effect of using the mean of initial and final weights rather than average

weight by periods; this tended to increase slightly the numerical value of the productive energy estimate.

The use of the factor 5.66 Cal. per gram to compute the energy equivalent of protein gain may also be questioned in this connection. Since the net energy value of tissue protein when oxidized should be no more (and probably less) than its metabolizable energy, it may be incorrect to credit protein with its full heat of combustion in computing energy gain of growth. Making the simplifying assumption that the end product of tissue protein oxidation would be uric acid, the heat of combustion of which is 8.22 Cal. per gram of nitrogen, the metabolizable energy yield per gram of tissue protein may be estimated at $5.66 - 0.16 \times 8.22 = 4.34$ Cal. per gram. The effect of using this factor to compute energy gain instead of 5.66 is also shown in table 5, for both the linear and exponential expressions of body weight. This results in a reduction of the numerical value of the productive energy estimate in every case, so the large difference between productive and metabolizable energy is evidently not due to use of the factor 5.66 for protein energy.

Although these alternative methods of computing productive energy give no indication of a reason for the large apparent SDA factor in energy utilization by the chick, it is of interest to note their effect on the variation as well as the magnitude of productive energy estimates. Using the average of initial and final weights instead of average weight by periods increased productive energy value slightly. It also increased variability as measured by the coefficient of variation, substantially when a linear expression of weight was used and slightly when an exponential expression was used. Using the factor 4.34 instead of 5.66 Cal. per gram of protein increased variability slightly as it reduced the numerical value of productive energy. Employing the exponential expression for relating maintenance need to body weight reduced the variation substantially (3 to 6%) and also reduced size of the productive energy estimate.

If the large difference between productive energy and metabolizable energy is indeed a valid estimate of SDA, this factor is a large part of the total heat production of the chick. If, as would seem more likely, productive energy is a parameter of net energy rather than a true estimate of it, the numerical value of the difference between productive and metabolizable energy would have little meaning in itself. Its biological significance, as well as that of the productive energy estimate itself, is uncertain.

It is also of interest to note that the mean productive energy value obtained in this work, 2.58 Cal. per gram, is approximately 20% greater than estimated for this diet from the data of Fraps for the diet components. The greater productive energy observed in these studies may reasonably be considered to be due to better nutritive balance and greater adequacy in micronutrients of our diet in comparison to those used in the earlier studies of Fraps.

These experiments indicate that metabolizable energy is the measure of choice for chicks with respect to accuracy. Whether it is a valid measure of metabolically useful energy, equally as valuable as some measure of net energy, will be the subject of further experiments.

SUMMARY

Experiments have been conducted to compare the determination of metabolizable and productive energy of a semipurified reference diet for the growing chick.

Metabolizable energy was found to be independent of plane of food intake in the range from 100 to 30% of ad libitum intake. Replicate determinations gave a mean value of 3.34 Cal. per gram of dry matter with standard deviation $\pm$ 0.04.

In contrast, replicate determinations of productive energy were highly variable, ranging from 2.06 to 3.31 Cal. per gram with a mean of 2.49 $\pm$ 0.42. From a regression analysis of all available data, its mean productive energy was 2.58 Cal. per gram. No consistent relationship between plane of food intake and productive energy value was found.

The observed productive energy was approximately 20% greater than estimated from the productive energy data of Fraps for the components of the diet. This was considered to be due to the superiority of the diet used in the present work as compared to the earlier studies of Fraps.

Productive energy in the present experiments was approximately 77% of metabolizable energy. The large difference between the two measures was not diminished by changes in method of computation involving the relation between body size and maintenance requirement or the energy equivalent of tissue protein. Its possible significance was discussed.

LITERATURE CITED

AXELSSON, J., AND S. ERIKSSON 1951 Determination of metabolizable energy in poultry foodstuffs and rations. Proceedings Ninth World's Poultry Congress, *2:* 160–162.

CARPENTER, K. J., AND K. M. CLEGG 1956 The metabolizable energy of poultry feeding stuffs in relation to their chemical composition. J. Science of Food and Agriculture, *7:* 45–51.

COULSON, E. J., AND J. S. HUGHES 1930 Collection and analysis of chicken urine. Poultry Science, *10:* 53–58.

DANSKY, L. M., AND F. W. HILL 1952 Application of the chromic oxide indicator method to balance studies with growing chickens. J. Nutrition, *47:* 449–459.

FRAPS, G. S., E. C. CARLYLE AND J. F. FUDGE 1940 Metabolizable energy of some chicken feeds. Texas Agr. Expt. Station Bull. 589.

FRAPS, G. S. 1944 Digestibility of feeds and human foods by chickens. Texas Agr. Expt. Station Bull. 663.

———— 1946 Composition and productive energy of poultry feeds and rations. Texas Agr. Expt. Station Bull. 678.

HALNAN, E. T. 1951 The nutritive (energy) value of poultry feeds. Proceedings Ninth World's Poultry Congress, *2:* 3–6.

KATAYAMA, T. 1924 On the digestibility of feedstuffs by chickens. Bull. Imperial Agr. Expt. Station Japan (Tokyo), *3:* 1–78.

MCKITTRICK, D. S. 1947 The selection of chicks for growth experiments and the evaluation of growth. Growth, *11:* 89–100.

OLSSON, N. 1950 Digestion experiments on poultry. Kungl. Lantbrukshögskolan och Statens Lantbruksförsök Statens Husdjursförsöle. Meddelande Nr. 43.

SCHÜRCH, A. F., L. E. LLOYD AND E. W. CRAMPTON 1950 The use of chromic oxide as an index for determining the digestibility of a diet. J. Nutrition, *41:* 629–636.

SWIFT, R. W., AND C. E. FRENCH 1954 Energy metabolism and nutrition. The Scarecrow Press, New Brunswick, N. J.

TITUS, H. W. 1955 The Scientific Feeding of Chickens. The Interstate, Danville, Illinois.

ADDENDUM

The work of J. Davidson, I. McDonald and R. B. Williams described in a paper entitled "The utilization of dietary energy by poultry. I.-A study of the algebraic method for determining the productive energy of poultry feeds" (J. Sci. Food Agric., *8:* 173–182, '57) came to our attention after the preparation of our manuscript. In their studies the productive energy determination was also highly variable, and they obtained values for simplified rations which were 10 to 40% lower than those predicted by Fraps' data. They concluded that the assumptions underlying the method are invalid, and that the values obtained are unreliable.

(56)

The Addition of Water to Purified Diets and its Effect upon Growth and Protein Efficiency Ratio in the Rat

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Preliminary studies in our laboratories indicated that the level of water in a purified diet may influence the growth rate and feed efficiency of rats fed suboptimal protein diets (9%).

Although several workers (Laguna and Carpenter, '51; Lepkovsky and Furuta, '60; Cizek, '59) have studied the effect of water addition to diets, a review of the literature indicated a dearth of information relating to the effect of water addition on protein evaluation.

A method proposed by Derse ('58, '60) for the evaluation of protein by means of the protein efficiency ratio (PER)[1] seems to be gaining widespread use.

However, Morrison and Campbell ('60) have shown that the length of time on experiment, the strain of the experimental animals and the level of protein used have a significant effect upon the PER; but these PER studies were conducted with diets containing low levels of moisture. To gain a better understanding of the effect of moisture in the evaluation of the protein quality of foods, it was decided to determine the influence of moisture level in a purified control diet. A more thorough investigation, therefore, was undertaken to determine whether the PER of a standard 9% casein purified diet would be influenced by the addition of various levels of water.

METHODS

Twenty-one-day-old male rats, descendants of the Sprague-Dawley strain,[2] were used in all experiments. The animals were individually housed in screen-bottom cages kept in an air-conditioned room maintained at 74 to 76°F. Food and water were supplied ad libitum. Daily records were kept of the amount of food consumed by each rat. The animals were weighed ind vidually at weekly intervals. The test die were prepared by the addition of water the dry basal diet as follows: 200 gm water plus 800 gm of diet (20% of wat added); 500 gm of water plus 500 gm diet (50% of water added); and 800 g of water plus 200 gm of diet (80% water added). The moisture and prote content of the diets were then determin directly. Diets were stored in airtight co tainers in the refrigerator to minimi possible moisture loss. In all experimen high nitrogen casein[3] was used as t source of protein.

Diet thickener studies

In preliminary trials it was noted th when various food products having a rel tively high (60 to 80%) moisture conte were used to prepare diets containing 9 of protein, the resulting diet was qui homogeneous. There did not appear to diet separation or settling out of solid But it was noted that when a purifi control diet (containing the same mo ture level) was prepared, the diet oft separated. It was thought advisable, the fore, to investigate not only the effect moisture level using a purified control di but also the effect of moisture level wi this same diet containing certain co pounds that could be used to yield a co sistency similar to that obtained when food product was used as the source protein. Diets containing high amoun

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[1] Grams gained per gram of protein consumed.

[2] S. and K. Animal Company, Jamesburg, N Jersey.

[3] ANRC, High Nitrogen Casein-30 mesh, Sheff Chemical Division, Norwich, New York. This ca has been approved by the Animal Nutrition Res Council for use in biological assay for nutritive v of protein-containing material.

of moisture and no diet thickener were stirred frequently in the food cups and the food allowed to remain until the cup was empty. This was done to be certain that any solids settling out were eaten, and that the animal was not merely consuming the liquid portion of the diet.

The composition of the diet is shown in table 1. This diet supplied 9% of protein on a solids basis. The diet thickeners, agar and guar gum, were added at a level of 0.5% of the diet at the expense of cellulose. The cornstarch was added at a level of 25% at the expense of sucrose. This was gelatinized by adding a small amount of cold water to the starch, then adding this mixture to boiling water. The gelatinized starch was cooled immediately to room temperature in a cold water bath and the remaining diet ingredients were then added.

Five rats were placed in each of the groups and the experiment was replicated three times. The combined results from the three replications were subjected to appropriate statistical analysis (t test, Snedecor, '50). The PER values obtained are summarized in table 2. The results showed that in the absence of any diet thickener, the addition of water at a level of 20% yielded a highly significant increase ($P < 0.01$) in PER over that obtained when no water was added to the diet. It was also found that in the presence of agar, guar gum or starch the addition of water at levels of 20 or 50% resulted in a highly significant increase ($P < 0.01$) in PER over that obtained when no water was added. Furthermore, the use of starch at a moisture level of 20% yielded a highly significant increase ($P < 0.01$) in PER over that obtained when agar was used. At the 80% moisture level, the solids settled out and the variation within groups was so great that no significant difference between groups could be detected. It was observed that when 20% of water was added to the diet the animals consumed more protein than when no water was added. To determine whether the increased PER was the result of increased protein intake, analysis of covariance (Snedecor, '50) was determined. The individual values of all animals that had received the zero or 20% water addition were pooled and analyzed (protein consumed versus PER). An F value of 68.5 ($P < 0.01$ with 1 and 117 degrees of freedom) was obtained. The difference between the PER's at zero and 20% water addition was still highly significant after being adjusted to a common protein intake.

TABLE 1

Composition of diet

	gm/100 gm
Casein[1]	10.16
Sucrose	71.64
Corn oil	8.00
Vitamin mix	0.10
Choline chloride	0.10
d α-Tocopheryl acid succinate	100 units
Cellulose	5.0
Mineral mix[3]	5.0
Vitamin mix	
Thiamine·HCl	0.500
Riboflavin	0.800
Niacin	4.000
Pyridoxine·HCl	0.500
Ca D-pantothenate	4.000
Biotin	0.040
Folic acid	0.200
Menadione	0.500
Cyanocobalamin	0.003
i-Inositol	10.000
p-Aminobenzoic acid	10.000
Vitamin A palmitate[2]	2000 IU
Vitamin D_2[2]	200 IU
Cornstarch to make	100.000

[1] ANRC, High Nitrogen Casein—30 mesh, Sheffield Chemical Division, Norwich, New York.
[2] Myvax, dry vitamin A palmitate with vitamin D_2, Distillation Products Industries, Rochester, New York.
[3] Jones and Foster ('42).

In the second experiment the effect of moisture level on PER was again studied at the 9% level of protein in the absence of a diet thickener, using 10 rats per group. The results of this experiment are shown in table 3. These results again showed that the addition of 20% of water to the basal diet yielded a highly significant increase ($P < 0.01$) in the PER over that obtained when no water was added to the diet. Covariance analysis was again determined between protein consumption and PER for rats that had received zero and 20% of water. An F value of 71.9 ($P < 0.01$ with 1 and 17 degrees of freedom) was obtained.

Since the possibility existed that the increased weight gain in animals that had received the moisture-containing diets was due to storage of water, the animals were

TABLE 2

Average results obtained in diet thickener studies

Water added to diet	Protein consumed	Weight gain	PER[1]	Protein consumed	Weight gain	PER
%	gm	gm	Diet thickener	gm	gm	
		None			Agar	
0	19.40	47.3	2.43±0.08	18.85	45.9	2.43±0.11
20	24.10	73.6	3.05±0.08	24.67	73.7	2.99±0.07
50	17.42	45.8	2.62±0.09	24.19	71.1	2.94±0.08
80	—	—	—[2]	18.84	49.1	2.66±0.22
		Guar gum			Cornstarch	
0	22.07	55.5	2.51±0.13	20.50	49.4	2.40±0.16
20	25.49	78.9	3.09±0.06	23.70	80.3	3.38±0.06
50	23.82	76.9	3.22±0.07	24.80	75.6	3.04±0.07
80	13.93	16.4	1.17±0.14	17.10	43.8	2.56±0.13

[1] Protein efficiency ratio, mean value with its standard error.
[2] In two of three replicates the animals failed to gain weight.

TABLE 3

Effect of moisture level on PER[1] at a protein level of 9% in the absence of diet thickener

Water added to diet	0	20	50	80
%				
Initial weight, gm	40.1	40.1	40.1	40.1
Avg weight gain, gm	52.4 ±2.8[2]	77.5 ±2.9	9.0 ±1.2	14.7 ±4.3
Avg of protein consumed	22.61±1.33	22.91±0.61	10.44±0.37	10.82±0.66
Avg PER	2.33±0.10	3.37±0.07	0.84±0.09	1.23±0.30

[1] Indicates protein efficiency ratio.
[2] Standard error.

TABLE 4

Carcass analysis

Water added to diet	Avg solids	Avg fat	Avg crude protein	Avg ash	Avg NFE[1] (by difference)
%	%	%	%	%	%
0	33.0	10.1	18.1	3.3	1.4
20	34.5	12.1	17.9	3.4	1.7
50	32.3	9.3	18.0	3.9	0.6
80	30.6	8.7	17.1	4.0	0.7

[1] NFE indicates nitrogen-free extract.

killed and subjected to carcass analysis. The results are presented in table 4. These indicate that the increased weight gain of the animals that had received 20% of moisture was true gain and not water accretion.

Protein level studies

To determine whether the effect of moisture addition on PER could be obtained at levels of protein above and below 9%, five 21-day-old male rats were fed diets containing 6, 9, 12 or 18% of protein to which was added zero, 20, 50 or 80% of wate The basal diet was similar to that prev ously used, with the exception that t sucrose and high nitrogen casein we varied to obtain the protein level desire As in the diet thickener studies, the expe ment was replicated three times with rats per group. The combined results fr the three replications were subjected statistical analysis as before.

The protein efficiency ratios obtain are presented in table 5. These resu

TABLE 5

Average PER[1] values obtained in protein level studies

Water added to diet	Protein consumed	Weight gain	PER[2]	Protein consumed	Weight gain	PER
%	gm	gm		gm	gm	
		6% protein			9% protein	
0	10.68	16.66	1.56 ± 0.10	22.23	52.1	2.34 ± 0.06
20	12.36	32.2	2.61 ± 0.11	24.60	79.93	3.25 ± 0.08
50	—	—	—[3]	16.45	32.2	1.95 ± 0.20
80	—	—	—[3]	12.69	13.05	1.02 ± 0.16[4]
		12% protein			18% protein	
0	40.59	106.7	2.62 ± 0.09	68.42	162.3	2.37 ± 0.04
20	41.24	121.6	2.95 ± 0.07	66.08	163.5	2.47 ± 0.05
50	29.80	57.8	1.93 ± 0.10	51.62	114.3	2.21 ± 0.07
80	21.17	36.8	1.73 ± 0.17	34.72	72.9	2.09 ± 0.09

[1] Indicates protein efficiency ratio.
[2] Mean value with its standard error.
[3] Animals did not grow.
[4] Mean of two replicates, in one case animals did not grow.

show that at protein levels of 6 and 9%, the addition of 20% of water yielded a highly significant increase ($P < 0.01$) in PER over that obtained when no water was added. At the 12% protein level the addition of 20% of water yielded a significant increase ($P < 0.05$) in PER over the same diet with no water added. At 50 and 80% of water, protein efficiency ratios were significantly less than at zero and 20% of water at protein levels of 6, 9 and 12%. But when the diet contained 18% of protein, the addition of 20% of water did not significantly increase the PER. The addition of 50 and 80% of water caused a highly significant decrease ($P < 0.01$) in PER from that obtained with the addition of zero or 20% of water.

Further examination of the data revealed that when no water was added to the diet, increasing the protein level from 6 to 9% or 9 to 12% gave a highly significant increase ($P < 0.01$) in PER. When 20% of water was added to the diet, a highly significant increase ($P < 0.01$) in PER was obtained when the protein level was increased from 6 to 9%. However, there was no difference between the PER obtained at 9 and 12% of protein. The relatively high variation within groups at the 50 and 80% moisture levels precluded statistical differences between the various levels of protein. Covariance analysis was determined between protein consumed and PER of rats that had received zero and 20% of moisture addition at a protein level of 9%. An F value of 54.4 ($P < 0.01$ with 1 and 27 degrees of freedom) was obtained.

DISCUSSION

The reason(s) for the increase in growth rate and PER when water is added to a purified diet is not known. From the data obtained by covariance analysis and because a significant increase in PER is not always accompanied by a significant increase in protein consumption, it is highly improbable that the amount of protein consumed is the causative factor.

Laguna and Carpenter ('51) reported that when water was added to their experimental diets, a highly significant growth promoting effect ($P < 0.01$) was obtained over that found when water was not added. Lepkovsky and Furuta ('60) have reported studies with the chick indicating that the nutritional value of a stock mash for chicks may be improved by treatment with water. Lepkovsky et al. ('60) have also reported that feeding with or without water does not affect the proteolytic activity of the intestinal contents. Cizek ('59), in studies of the relation between water and food consumption, suggested that the osmotic pressure of the diluted food contents in the gastrointestinal tract may play an important role. Harper and Spivey ('58) have shown that the capacity of solutions of different dietary carbohydrates to exert osmotic pressure can influence the

protein intake of rats receiving low-protein diets sufficiently to affect growth. The growth stimulation observed in some of these experiments, however, may be due to factors different from those responsible for the effect noted when moisture is added to purified rat diet.

In view of these results, the moisture content of the control diet in PER evaluations may be of more importance than previously thought. Consequently, it would seem advisable to re-examine PER studies that have been made without taking this factor into consideration.

Further studies using various carbohydrate and protein sources in the presence and absence of water additions are in progress.

SUMMARY

The data reported here demonstrated that the addition of 20% of water to a 9% protein purified diet used for protein efficiency ratio (PER) evaluations resulted in a significantly increased rate of gain and a higher PER than that obtained when no water was added to the diet. These effects were also observed at protein levels of 6 and 12% but not at 18%.

When either agar, guar gum or gelatinized starch were included in the 9% protein diet, the addition of 20 or 50% of water resulted in an increased rate of gain and a higher PER.

The results observed in these experiments emphasize the need for further investigation of the factors involved i determination and a possible re-eval of previous studies wherein abnormal ture content may have been observe

ACKNOWLEDGMENTS

The authors wish to thank James lins and Joseph Conte for diet prepar as well as care and feeding of the an

LITERATURE CITED

Cizek, L. J. 1959 Long term observat relationship between food and water in in the dog. Am. J. Physiol., *197:* 342.

Derse, P. H. 1958 Evaluation of protei ity. II. Methods. J. A. Offic. Agr. Che 192.

——— 1960 Evaluation of protein (biological method). Ibid., *43:* 38.

Harper, A. E., and H. E. Spivey 1958 Re ship between food intake and osmotic ef dietary carbohydrate. Am. J. Physiol. 483.

Jones, J. H., and C. Foster 1942 A salt m for use with basal diets low or high in phorus. J. Nutrition, *24:* 394.

Laguna, J., and K. J. Carpenter 1951 versus processed corn in niacin deficient Ibid., *45:* 21.

Lepkovsky, S., and F. Furuta 1960 The of water treatment of feeds upon the nutr value of feeds. Poultry Sci., *39:* 390.

Lepkovsky, S., A. Chari-Bitron, R. L. Lyma M. K. Dimick 1960 Food intake, water and body water regulation. Ibid., 39: 39

Morrison, A. B., and J. A. Campbell Evaluation of protein in foods. V. Facto fluencing the protein efficiency ratio of J. Nutrition, *70:* 112.

Snedecor, G. W. 1950 Statistical Methods Iowa State Collegiate Press, Ames.

(8) That the detection of agar, tragacanth, karaya, guar flour, psyillum seed, and oat flour gums in frozen desserts be studied collaboratively.

(9) That methods for separation of mixtures of gums be investigated.

(10) That quantitative methods for gums in frozen desserts be investigated.

(11) That work be continued on the detection of alginates and other stabilizing agents in dressings for foods.

(12) That the spectrophotometric method for detection of stabilizers in soft curd cheese, as described by the Referee in 1954 [*This Journal*, 38, 189 (1955)] be studied collaboratively.

Isolation and Detection of Gums in Frozen Desserts*

By J. A. McNULTY (Food and Drug Administration, 1401 S. Hope St., Los Angeles 15, Calif.)

The proposed promulgation of definitions and standards of identity for frozen desserts (1) dictates that increased attention be given to the detection and determination of gums in frozen desserts. This topic has been under consideration by this Association for 25 years. Cross (2) reported that there were no suitable methods at that time (1934) and undertook a study of gums in various products, including ice cream. The first report to the Association of a method that showed promise was made by Hart (3) who reported some success with a method for gums in cheese. He detected the presence of gums in authentic ice creams containing locust bean and tragacanth. This method was not studied collaboratively.

Official methods are available for the isolation and detection of gums in soft curd cheese, and in mayonnaise and French dressing (4, 5). With these methods the presence of gums has been detected by reactions of hydrolysis products after the gum has been isolated. Essentially, this isolation technique consisted in removal of fat with ether, precipitation of proteins, and final precipitation of the gum from aqueous solution with alcohol. Experiments conducted by previous Associate Referees showed the procedure would work on ice cream, but results of collaboration were poor. Failures to recover the gum material were frequent, and losses probably occurred when emulsions formed during fat extraction (6). Johnson (7) reported a method for isolating gums in cheese spread in which the problem of emulsions was overcome by using dioxane, which has the property of removing fat and water. This method had not been subjected to collaborative study. Using dioxane to remove butterfat from a laboratory ice cream worked exceptionally well when tried by the Associate Referee.

Chemical methods for identification of gums have been published (8, 9). Newburger, *et al.* (10, 11) published a technique for obtaining the infrared spectra of water-soluble gums. The latter method was chosen for study because of the specificity offered by the technique. A thin film of the gum is prepared and the infrared spectrum, in air, is determined.

Essentially, the method of isolation involves removal of fat with dioxane, precipitation of protein, and isolation and purification of the gum by alcohol precipitation. Obviously the gum must be rather pure

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for identification and, for this reason, the method employs a triple precipitation as cleanup. Dilute HCl (1 + 1) is used instead of acetic acid (4, 5) because carbonyl-type absorption is characteristic of gums. "Salting out" is accomplished with saturated NaCl solution instead of $KAl(SO_4)_2$ solution because NaCl is transparent in the infrared while ionic sulfate shows absorption in the 9 μ region. In all probability these interferences would be present in small amounts; however, time did not permit a study of the effects that might be produced in the spectra.

METHOD

Reagents

(a) *Dioxane.*—Technical grade is satisfactory. (*Caution:* Vapors are obnoxious and harmful.)

(b) *Trichloroacetic acid solution.* — 50%. Freshly prepared as needed from non-hydrolyzed reagent.

(c) *Organo-silicone compound.*—To prepare non-wettable surface. Desicote ® (Scientific Instruments Division, Beckman Instruments, Inc., Fullerton, Calif.) has been found satisfactory.

Apparatus

(a) *Infrared spectrophotometer.*—Recording, for operation in 2–15 μ region.

(b) *Water-repellent plate.*—Wash 3–4" glass square thoroughly with detergent, rinse, and dry with towel. Dip glass rod in organo-silicone compound and streak adhering liquid across plate. Repeat streaking several times. Rub plate with lens paper to distribute evenly; then rub with clean lens paper to remove excess. Plate is now ready for use. Wash plate with cold H_2O and dry with towel after each use. Plate can be reused as long as it remains nonwettable.

Preparation of Sample

Weigh 50 g sample of frozen dessert into 250 ml centrifuge bottle and heat to 60° in H_2O bath. Add 150 ml dioxane, shake vigorously 2 minutes, and centrifuge 10 minutes at 1800 rpm. Decant and discard supernatant. Add 30 ml ether and shake vigorously to break mass at bottom, using rod if necessary. Decant ether and repeat ether wash twice. Heat in H_2O bath to remove residual ether. Add 30 ml 80° H_2O and shake vigorously 2 minutes to dissolve or disperse residue.

Separation of Gum

Add 20 ml 50% trichloroacetic acid and heat to 60° in H_2O bath. Shake 1 minute and centrifuge 10 minutes at 1200 rpm. Decant solution through fast folded paper into second centrifuge bottle, and discard residue.

Fill centrifuge bottle with alcohol, add 1 ml saturated NaCl solution, mix, and let stand until coagulation occurs. If precipitate does not form, gums are absent. (Let opalescent solutions stand overnight to facilitate precipitation. Centrifuge and discard if no precipitate is present.) Centrifuge 10 minutes at 1800 rpm and immediately decant and discard supernatant.

Purify precipitated gum by adding 30 ml 80° H_2O and shake vigorously to dissolve or disperse precipitate. Fill centrifuge bottle with alcohol, add 1 ml saturated NaCl solution and 1 drop HCl (1+1), mix, let precipitate coagulate, centrifuge 10 minutes at 1800 rpm, decant, and discard supernatant. Repeat purification step twice.

Chemical Detection of Gum

(a) *Before infrared identification.*—Scrape small amount of precipitated gum from centrifuge bottle with spatula and transfer to 50 ml beaker with about 10 ml hot H_2O. Add 2 ml HCl and boil 5 minutes. Neutralize to multirange indicator paper, using 30% NaOH solution first and completing with 10% NaOH solution. Remove paper and continue as in 15.147, beginning ". . . add 5 ml Benedict's solution."

(b) *Testing large amount of precipitate.*—If infrared spectrum is not desired, spectrum indicates presence of 2 or more gums, or precipitated gum will not form film (as with karaya), proceed as in 15.147, beginning "Add to residue in tube . . ."

(c) *Infrared identification.*—(May not be applicable to mixtures of gums.) Dissolve or disperse residue in 35 ml H_2O. Prepare film as below, obtain infrared spectrum against air, and compare with spectra of reference gums.

Preparation of Gum Films

Place H_2O repellent glass plate over 2" opening on steam bath. Pour enough of aqueous gum solution on plate to form circle about 2" diameter. (Volume required to produce film of sufficient area to block sample beam and of suitable thickness to produce characteristic spectrum varies with nature of

gum and its concentration.) Heat plate until film is dry and remove film with forceps. If film sticks to plate, remove with razor blade or tissue lifter. Transfer film to beaker and dry at 100° for 15–30 minutes. (Excessive heating may char some gums.) Place piece of film between 2 salt plates and obtain infrared spectrum against air.

Preparation of Reference Gum Films

Disperse 0.2 g gum in 30 ml 80° H_2O and add 20 ml 50% trichloroacetic acid solution. Continue as in *Separation of Gum*, beginning "Shake 1 minute and centrifuge 10 minutes at 1200 rpm."

Collaborative Study

Each collaborator was sent seven bottles of dry mix, two tubes of butterfat, and seven reference gums (guar flour gum, locust bean gum, Irish moss, pectin, gum arabic, agar, and sodium carboxymethylcellulose). The dry mix contained 5.7 g nonfat dry milk solids, 6.0 g sucrose, and 1.5 g dextrose. Five of the bottles also contained 0.2 g of a gum equivalent to 0.4% gum in the collaborative ice cream mix. (The proposed standards permit 0.5% gum.) Two of the bottles were marked as practice samples: "With added gum" and "Without gum." The other five bottles were identified by number only. The collaborators were instructed as follows: "Transfer 6 g butterfat and one bottle of dry mix to a 250 ml centrifuge bottle. Wash the bottle with a total of 31 ml hot (80°) water, and transfer washings to the centrifuge bottle. Shake vigorously 2 min. Additional warming and shaking may be necessary to produce uniform emulsions. This will simulate 50 g ice cream. Proceed with analysis, beginning, "Add 150 ml dioxane . . ." [This mixture is within the limits described by Sommer (12)]. Collaborators were not asked to perform the chemical tests for presence of gums.

The procedure for obtaining films of the gum material isolated was editorially changed after the method was sent out for collaboration. Instructions were reworded to prevent ambiguity since one collaborator submitted results that showed he used the entire amount of gum isolated to prepare the films. These films showed complete absorption in some areas; however, the unknowns were correctly identified. Two collaborators had previously reported correct results with excellent spectra before the change was issued.

Results and Discussion

Spectra submitted by collaborators closely parallel those for locust bean, Irish moss, pectin, gum arabic, and sodium carboxymethylcellulose (see Figs. 1 and 2). Results of collaboration are shown in Table 1. Except for Collaborator 8 all collaborators isolated all gums and each identification was correct with the exception of collaborative Sample 3, which contained locust bean gum. Most collaborators stated that locust and

Table 1. Collaborative results for identification of gums in ice cream mix[a]

Sample	Gum Present	Collaborator								
		1	2	3	4	5	6	7	8	9
With added gum	Sodium carboxymethylcellulose	x	x	x	x	x	x	x	x	x
Without gum	—	x	x	x	x	x	x	x	x	x
No. 1	—	x	x	x	x	x	x	x	—[d]	x
No. 2	Arabic[b]	x	x	x	x	x	x	x	—[d]	x
No. 3	Locust bean[c]	Guar or locust	Guar or locust	Guar	x	x	Guar, possibly locust	x	Locust-guar	Guar
No. 4	Irish moss	x	x	x	x	x	x	x	x	x
No. 5	Pectin	x	x	x	x	x	x	x	x	x

[a] x = Correctly identified. [b] Sometimes called acacia gum. [c] Sometimes called locust seed gum, carob bean gum, or St. John's Bread. [d] See explanation under *Results and Discussion*.

gar gave practically identical spectra. This difficulty was to be expected. Newburger, *et al.* (11) report: "The spectra of locust bean and guar flour gums are indistinguishable. However, some authorities believe that these gums are identical or at most differ in the proportions of substances which they contain." Ewart and Chapman (8) report: "Locust bean and guar gums give identical reactions and cannot be distinguished on the basis of these (chemical precipitation) tests." The Associate Referee found that the two untreated gums had identical spectra, but treating the gums as in the proposed method produced marked differences in the 10 to 10.5 μ region. Guar gum (Fig. 1) showed a minor peak at 10.3 μ; locust bean (Fig. 1) produced a flat plateau between 10 and 10.5 μ. Collaboration disproved this as a means of differentiation. Some collaborators obtained films for locust bean that also showed a minor peak at 10.3 μ.

The samples marked "Without gum" and "No. 1" were identical. Collaborator 8 reported, "powdery precipitate formed which would not give a film" for Sample 1. It is presumed that this material was protein. Possibly not enough trichloroacetic acid was added or the temperature might have been too low to effect good coagulation of the protein. A gum was isolated by Collaborator 8 for Sample 2 and the spectra were submitted; however, no identification was made. He reports: ". . . films for the reference gums were satisfactory except in the case of acacia. The film could not be removed from the glass plate. The film chipped off in fine flakes and could not be used. This was also true of collaborative Sample 1. The film from collaborative Sample 2 was also difficult to handle."

Since this collaborator did not place his films between salt plates, but rather used a cardboard holder with windows, it is presumed this is the reason he did not submit a spectrum of reference acacia. The spectrum he submitted for collaborative Sample 2 could be superimposed on spectra submitted for Sample 2 by other collaborators who used the same model instrument as Collaborator 8.

One collaborator reported Sample 4 contained agar when it actually contained Irish moss. His reference spectra and the spectrum obtained from Sample 4 were given to an analyst in this laboratory who was unfamiliar with the collaborative study or the incorrect identification. This analyst correctly identified the spectrum of Sample 4 as Irish moss. For this reason the reference spectra and spectra obtained from Samples 3 and 4 were returned to the collaborator for re-evaluation. His original identification of Sample 3 as locust bean gum was correct. He subsequently identified Sample 3 as guar gum and Sample 4 as Irish moss. He reported choosing Irish moss on the basis of the spectral region between 10 and 12 μ. This was the same area used by the analyst in this laboratory to distinguish between agar and Irish moss.

The spectra obtained from films of untreated guar, locust bean, pectin, agar, and tragacanth gums (Figs. 1 and 2) do not differ materially from the spectra obtained from films of the gums recovered in the isolation and reference procedures. The only differences were in the relative heights of absorption peaks. Possibly this can be explained by the fact that gums are natural products and contain many compounds. Slight changes in technique between analysts may isolate more or less of a particular compound, giving rise to the differences in relative heights of absorption peaks. Gum arabic (Fig. 2) and psyllium seed gum (Fig. 2) produced essentially the same spectra for treated and untreated material except for slight changes in carbonyl-type absorption in the 6 μ region. Sodium carboxymethylcellulose (Fig. 2) varied considerably in the 5.5 to 8.5 μ region for the treated and untreated gums. The dotted areas indicate the trace for the untreated gums in Fig. 2 (arabic, sodium carboxymethylcellulose, and psyllium seed).

The collaborators were asked to report what type of device was used to hold the films in obtaining the spectra. Two used the conventional liquid cell holder (demountable type), and one a holder normally used in the mull techniques. Collaborator 8 placed his films between two pieces of stiff paper with a window cut to allow passage of the

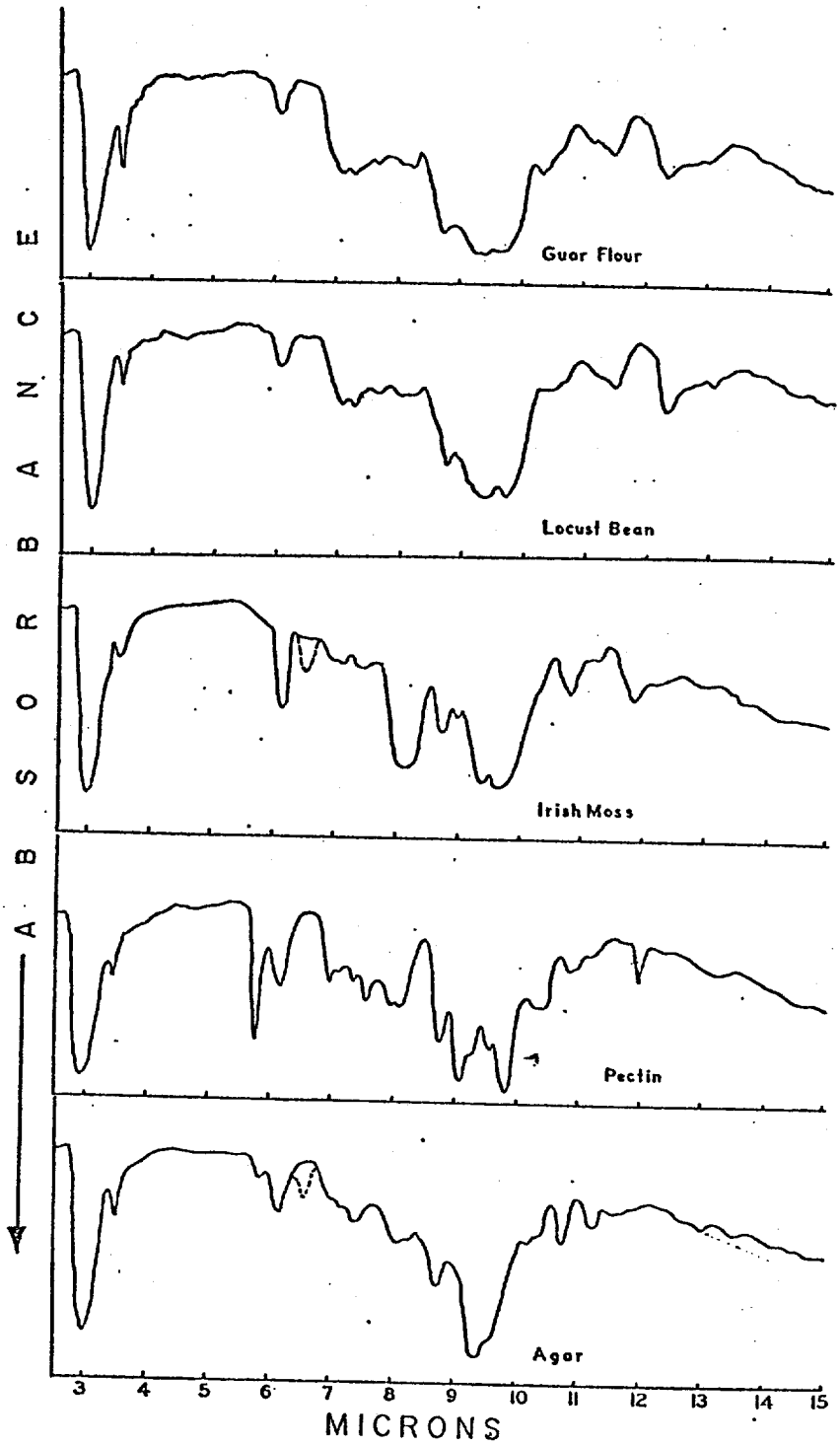

Fig. 1—Spectra of gums isolated by the method.

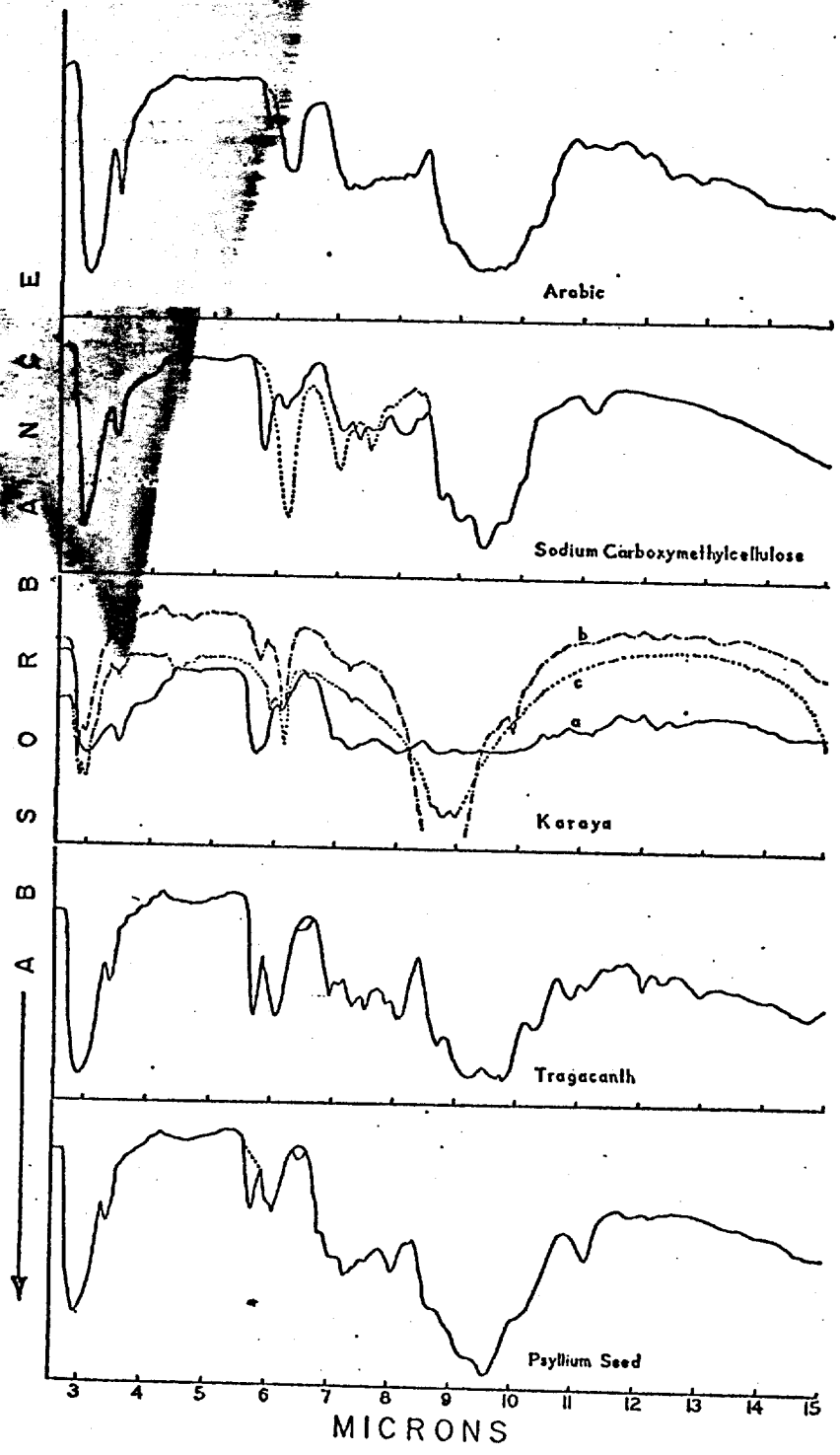

Fig. 2—Spectra of gums isolated by the method.

beam. While this technique is successful with well-formed films it cannot be used with the brittle, flake-type film such as gum arabic produces. The other collaborators used a device similar to the one described by the Associate Referee in his instructions. This device holds a piece of film between two salt plates with tension supplied by rubber bands. It is constructed of pressed board and eye screws. Samples can be changed quickly but it tends to damage portions of the film not covered by the salt plates. If no tension is put on the salt plates, films may fall out of the holder. Pressure may fracture brittle films, cause multiple reflections, and thereby give "jitters" to the recorder pen. While undesirable, this type of spectra does not negate identification since gums are natural products containing many compounds and, because of this, the absorption peaks are broad and an even pen line is not necessary for differentiating between spectra.

Experimental Work

A minor absorption peak at 6.5 μ occurred in each study of Sample 4, Irish moss, which did not appear in the spectrum of the reference gum. This absorption also occurs to a much smaller degree in agar, tragacanth, and psyllium seed gums isolated from the collaborative ice cream mix. (See dashed areas of Figs. 1 and 2). In order to study the nature of this peak the Associate Referee added Irish moss to butterfat, to sucrose, to dextrose, and to nonfat dry milk solids. The peak at 6.5 μ occurred only in the film of Irish moss isolated from the milk solids. A second protein precipitation did not remove this peak and produced an extremely poor, brittle film. The spectrum of isolated Irish moss that had been added to pure casein showed a minor absorption at 6.5 μ but not as intense a one as that from the milk solids. Conditions in this experiment were not exactly the same, however, since the casein was in large granules. It could not be reduced by grinding and, therefore, had to be dissolved in a 1% NH_4OH solution with the Irish moss. Irish moss was isolated from two other commercial brands of "instant" nonfat dry milk and these spectra also showed absorption at 6.5 μ. This type of study has not been conducted on agar, tragacanth, or psyllium seed gum.

A commercial "ice milk" frozen dessert was analyzed by the proposed method. The material isolated formed a film and the infrared spectrum was obtained (Fig. 3a). This spectrum was quite similar to that of tragacanth in the 8.5 to 11.5 μ region. From 11.5 to 12.5 μ the spectrum resembles guar or locust bean. The doublet effect between 5.5 and 6.5 μ occurs with about the same relative intensity as in psyllium seed and tragacanth. None of the other gums studied showed this doublet effect.

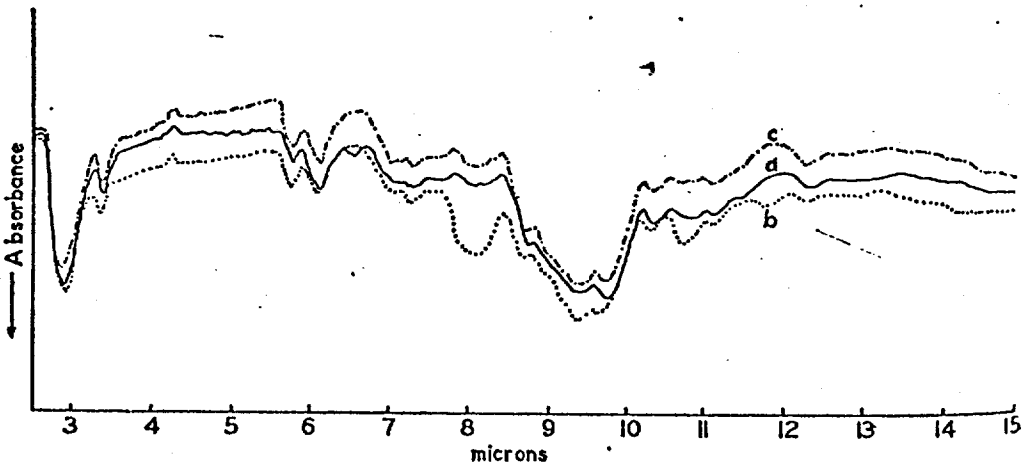

Fig. 3—Spectra of (a) gum material isolated from commercial "ice milk," (b) 1:1:1 locust bean, Irish moss, and sodium carboxymethylcellulose, (c) 1:1 locust bean and sodium carboxymethylcellulose.

The manufacturer of the ice milk said that the gum material used was supposed to contain a 1:1 mixture of locust bean and Irish moss to which he added sodium carboxymethylcellulose in an amount to make the final gum concentration a 1:1:1 mixture of sodium carboxymethylcellulose, locust bean, and Irish moss. A reference spectrum was prepared, using the 1:1:1 ratio (Fig. 3b). This spectrum showed strong absorption at 8 μ due to sulfate absorption of the Irish moss and slight absorption at 11.7 μ—also characteristic of Irish moss. A spectrum was prepared of sodium carboxymethylcellulose and locust bean in 1:1 ratio (Fig. 3c). This spectrum matched that of the gum material isolated from the ice milk. The strong absorptions at 8 μ and 11.7 μ were missing, indicating that no Irish moss was present in the ice milk. A sample of the commercial stabilizer used by this firm was obtained. The gum material was isolated and identified as either guar flour or locust bean gum. Information obtained about the formula for this stabilizing mix indicated it contained only locust bean gum and sucrose.

Chemical tests for the detection of gums were not studied collaboratively. The test with Benedict's solution (15.144(a)) for the detection of gums has been adopted as official (13). This test, in the modified form described in this report, was applied to Irish moss, tragacanth, psyllium seed agar, and the material isolated from the commercial "ice milk" dessert. Karaya isolated from the collaborative ice cream mix was tested by 15.147 (4). All tests were conducted by the Associate Referee and classified positive on the basis of the presence of a yellow, orange, or red precipitate.

The remaining gums permitted by the proposed standards, psyllium seed, guar, oat, tragacanth, karaya, agar, and the alginates were not subjected to collaborative study. The isolation and identification of these gums, with the exception of oat flour gum and the alginates, were studied by the Associate Referee. Each of the gums, except karaya, was isolated and each produced a film suitable for infrared identification (Figs. 1 and 2). Karaya, when isolated from the collaborative ice cream mix, produced a granular precipitate that formed a film with difficulty. The spectrum of this film was obtained (Fig. 2, Karaya b). This material was also ground and the spectrum was obtained by the KBr wafer technique (Fig. 2, Karaya c). These spectra were completely different from the spectra of the reference gum (Fig. 2, Karaya a). Karaya does not form a true solution, and most of the gum is probably lost in the protein precipitation step of the method. Hydrolysis may also be a factor. However, the presence of a gum in a frozen dessert containing karaya can be detected by 15.147 (4). Gelatin, also permitted in the proposed standards, is removed in the protein precipitation. A method for detecting alginates in chocolate products (14), including chocolate ice cream, has been adopted as official (15) by the Association. No oat flour gum was available to the Associate Referee for study.

Summary and Conclusions

A method has been described for the isolation, detection, and identification of gums in frozen desserts. The isolation and infrared identification of five singly occurring gums—locust bean, Irish moss, pectin, arabic, and sodium carboxymethylcellulose—was studied collaboratively with excellent results.

Agar, tragacanth, psyllium seed, and guar gum can also be detected and identified by the method, but they have not undergone collaborative study.

Guar flour gum and locust bean gum cannot be distinguished by this infrared technique. Karaya gum cannot be identified when isolated by the proposed procedure. The presence of a gum in a frozen dessert containing karaya (and/or other gums) can be detected by chemical tests. Brief study indicates that Irish moss, and to a lesser degree agar, psyllium, and tragacanth, binds some material from milk solids that cannot be precipitated by the protein precipitation method described. This material absorbs at 6.5 μ and is quite possibly nitrogenous in nature.

A commercial sample of frozen dessert was studied by the Associate Referee in which he detected the absence of one of the

gums reported to be a constituent of a mixture of three gums.

Recommendations

It is recommended[1]—

(1) That the method of isolation of gums in frozen desserts be adopted as first action.

(2) That the detection of agar, tragacanth, karaya, guar flour, psyllium seed, and oat flour gums in frozen desserts be studied collaboratively.

(3) That methods for separation of mixtures of gums be investigated.

(4) That quantitative methods for gums in frozen desserts be investigated.

Acknowledgments

The Associate Referee wishes to express gratitude to M. J. Gnagy, General Referee on Gums in Foods; and to J. T. Welch, former Associate Referee, for assistance in developing the method; to C. Comstock, Horton and Converse, Los Angeles, who supplied some of the gums used in the study; and to the following collaborators, all of the U.S. Food and Drug Administration: T. E. Byers, E. C. Pranskaitis, P. S. Jorgensen, B. W. Rehn, J. F. Weeks, C. E. Beisel, S. Nesheim, L. F. Schneiderer, A. I. Kleks, and M. J. Gnagy.

[1] These recommendations were approved by the Referee and Subcommittee C and were adopted by the Association. See *This Journal*, 43, 130 (1960).

References

(1) *Federal Register*, 23, No. 60, March 26, 1958, pp. 2008-2010.
(2) Cross, L. J., *This Journal*, 17, 380 (1934)
(3) Hart, F. L., *ibid.*, 20, 527 (1937).
(4) *Official Methods of Analysis*, 8th Ed., Association of Official Agricultural Chemists, Washington, D.C., 1955, 15.144-15.147.
(5) *Ibid.*, 28.49 (a) and (b).
(6) Gnagy, M. J., *This Journal*, 38, 189 (1955).
(7) Johnson, R. H., *ibid.*, 39, 286 (1956).
(8) Ewart, N. H., and Chapman, R. A., *Anal. Chem.*, 24, 1460 (1952).
(9) *Official Methods of Analysis*, 8th Ed., Association of Official Agricultural Chemists, Washington, D.C., 1955, 32.286-32.289.
(10) Newburger, S. H., Jones, J. H., and Clark, G. R., *Proceedings of the Scientific Section of the Toilet Goods Association*, Number 18, December 1952.
(11) Newburger, S. H., *et al.*, *ibid.*, Number 19, May 1953.
(12) Sommer, H. H., *Theory and Practice of Ice Cream Making*, 3rd Ed., 1938, pp. 10 and 11.
(13) Report of Subcommittee C, *This Journal*, 34, 50 (1951).
(14) Perlmutter, S. H., *ibid.*, 40, 478 (1957).
(15) Report of Subcommittee C, *Ibid.*, 42, 22 (1959).

Natural Plant Hydrocolloids — Part II

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This is the second in a series of articles dealing with the "natural plant hydrocolloids" commonly known as "water soluble gums".

This paper will discuss agar, gum karaya, gum guar, Irish moss and Irish moss extracts, and describe the history, origin, physical and chemical characteristics as well as the general uses of these gums. The first article discussed gum tragacanth, gum arabic and locust bean gum (see American Perfumer, February, 1962).

It is worth noting that due to the advances in the processing and standardization of these natural gums, it becomes increasingly important that the user of these products familiarize himself with the latest techniques of processing and quality control. The analyses routinely performed in a modern natural gum laboratory are: identity, viscosity and/or gel strength, mesh analysis (US standard screens), pH, insoluble matter, moisture, total ash, acid insoluble ash and a microbiological analysis where required.

The third article in this series will discuss the hydrocolloids most suitable for a given application, and will offer basic formulations for preparing such products as emulsions, jellies, lotions, toothpaste, wave set, etc.

Agar

Identity and Origin: Agar, or agar-agar as it is sometimes known, is the dried extract from *Gelidium, Gracilaria* and other related species of red algae, which are seaweeds growing in wide areas, on the rocks and in the sea. It is a unique member of the plant hydrocolloids in that it has a low gelling and a high melting temperature. Commercial sources are Spain and Japan.

Agar has been known to commerce since 1870. Agar is available in many forms: in thin strips packed in bundles, in flake form and as a powder.

Physical characteristics: Unground agar is packed in bundles from 30 to 40 cm. long of thin translucent strips 0.5 to 1 cm. in width.

Powdered agar is creamy-white in color and practically odorless and tasteless.

Solubility: Agar is insoluble in cold water; slowly soluble in hot water; easily soluble in boiling water.

Agar is insoluble in alcohol and organic solvents.

Viscosity: Agar forms sols whose relative viscosity above the melting temperature is rather low. Heat, pH and presence of electrolytes affect the viscosity of agar sols.

Agar swells rather slowly in water, the swelling takes place in 30 minutes. Its maximum swelling rate is at pH 8 to 9. It tends to swell less in acid media than in alkaline media. The water absorbing ability of agar gradually increases as the water content decreases up to a certain point. Agar in 1% concentration forms a strong gel on cooling below 40°C.

Chemical characteristics: Agar is believed to be a linear polygalactose sulfuric ester. In solution it forms an anionic electrolyte with negative charges on each polymeric unit. The important cation is usually calcium, although magnesium, sodium and potassium are also present.

Powdered agar contains 15 to 18% moisture, and 0.5% acid insoluble ash.

pH: The pH of a 1% sol is neutral. The pH affects the relative swelling character of the gel, apparently reaching its maximum at 8 to 9.

Preservatives: Agar is similar to other plant hydrocolloids in that it is subject to microbial attack. Methyl and propyl parabens in 0.2% and 0.05% concentration respectively are effective.

Compatibility: Agar is compatible with other plant

hydrocolloids such as locust bean gum as well as carbohydrates and proteins.

Electrolytes reduce the swelling power of agar as do alcohol and other water miscible organic solvents. The action is two-fold: to reduce the net electric charge and to dehydrate the gel. The electrolytes at 0.1N concentration and the colloid compete for the water present. This action is reversible.

Gel Formation: The ability of agar to form strong gels with a sharp transition temperature is its most important property. An agar sol obtained by heating to 95°C. will not solidify till the temperature drops to about 36° to 40°C. The gel thus formed may be treated with hot water without dissolving below 95°C. Hard gels are formed in less than 1% concentration. A range of gel strengths are available depending on end use requirements. The gel strength is nearly directly proportional to the concentration of Agar used between 0.5% and 2.0%.

When locust bean gum is incorporated, the elastic deformability and breaking strength are greatly increased. The gel strength of agar diminishes with age.

As a rule agar gels shrink and exude some liquid from their surface (syneresis). Agar shows decreasing syneresis with rising concentration.

Uses: The high degree of hysteresis as well as the great swelling power of agar and it its ability to form hard gels are its important properties. It is also indigestible and, therefore, non-nutritive. Agar acts more quickly than gelatin but has a low whipping point. Pharmaceuticals: bulk laxatives; dental impressions. Cosmetics: in special emulsions. Microbiological: culture media and nutrient broths.

Gum guar

Identity and Origin: Gum guar is processed from a leguminous plant. *Cyamopsis Tetragonoloba*, which resembles the soybean plant. The bean pods grow along the vertical stem of the plant, which is grown extensively in Pakistan and also in the United States, mainly as cattle feed.

The bean pod, 6 inches long contains 5 to 6 seeds, considerably smaller than the locust bean gum seeds. Roughly 14-17% of the seed is the hull, 35-42% represents endosperm and 43-47% germ.

Physical Characteristics: Guar flour is nearly white; its color depending on the mesh size. The technical grades are darker in color.

Solubility: Gum guar will swell almost completely in cold water. It will hydrate to a practically colorless sol. It is insoluble in organic solvents.

Viscosity: Powdered gum guar can be dispersed rapidly in water. The rate of hydration, however, is directly proportional to the temperature of the water, as well as particle size. At 80°C the maximum viscosity development occurs in 6 to 10 minutes. The rate of swelling is affected by pH as well as dissolved substances. The viscosity of a 1% sol is 2700 cps. Gum guar sols are stable towards heat (100°C) for long periods of time. Guar sols are thixotropic.

Chemical Characteristics: Gum guar is essentially a polysaccharide composed of a straight chain of D-mannose with a side chain of one D-galactose on approximately every other mannose unit. The molecular weight is on the order of 220,000. There is a 2:1 ratio of mannose to galactose in guar, while almo a 4:1 ratio in locust bean gum.

Gum guar, on the average contains 10% to 1 moisture, 5% to 7% protein, 0.5% acid insoluble re due with an approximately 80% galactomannan co tent.

pH: The pH of a 1% sol is between 5.5 and 6. It tends to become more acid on standing. Gum gu sols exhibit a buffering action on pH and are stal over a pH range of 4 to 10.5. Extreme acid and alk line conditions beyond these limits will virtually st the rate of hydration of the sol. However, if the gu is first hydrated at a moderate pH, more alkali m be added and sols with pH of 11 or more may prepared.

Preservatives: Guar sols are similar to other pla hydrocolloids in that they are subject to microbi attack. Benzoic acid (below pH 4), methyl and pr pylparabens, sodium o-phenylphenate at 0.1% conce tration, have been used successfully to preserve gu sols.

Compatibility: Gum guar is compatible with oth plant hydrocolloids such as agar, arabic, karaya, a tragacanth. Guar is also compatible with raw starch gelatin and other water soluble proteins.

The presence of dissolved salts and water miscil solvents alter the swelling rate and affect the fi viscosity of guar sols. Specific applications of co patibility can easily be determined. If guar is add to water of an alkaline pH containing small amou of borate ions, the gum will be inhibited and w not swell.

Gel Formation: The addition of small amounts borax to guar sols causes gelling. The borate ion a as a crosslinking agent and forms rubbery gels. Th gel can be reversed by adjusting the pH to less th neutral. The new sol will have the same viscos as the original sol.

Uses: Gum guar is extremely versatile: as a s pending agent, viscosity modifier, stabilizer, dispe ant and selective flocculant. Pharmaceuticals: appet depressant, peptic ulcer therapy, tablet disintegrat and binding agent. Cosmetics: lotions, creams, fa masks.

Gum karaya

Identity and Origin: Gum karaya, sometimes kno as Sterculia gum, is the dried exudation of the *St culia urens* tree and other species of *Sterculia*. T tree is native to India which is the sole source supply. The trees are now cultivated and the g production closely supervised. Sap oozes out of t dried hole and collects, forming lumps which wei up to 5 pounds. The quality of the gum as well the yield improves during the warm, dry weath The crude gum is brought to the Bombay market sorting into grades according to color and fore matter.

Physical Characteristics: The highest grade so of gum karaya are white, translucent and almost fr of adhering bark. The lower grades vary from li yellow to brown and may contain as much as 3 soluble impurities. Powdered gum karaya is li pink-gray in color.

Solubility: Gum karaya, like gum tragacanth, d

Tacket forms a colloidal sol.

Powdered gum karaya swells in cold water to an extent that a 3% to 4% sol will produce heavy gels of uniform smoothness and texture. For higher concentrations it is necessary to cook the gum under steam pressure in order to make it soluble. A 20% to 25% solution may be prepared in this manner. It yields a thick, syrupy-like, brown liquid. Gum karaya will form viscous sols in hydroalcoholic solutions ranging up to 60% alcohol, while gum tragacanth will hydrate in solution up to 35% alcohol concentration.

Chemical Characteristics: Gum karaya is a complex polysaccharide of high molecular weight. A molecular weight as high as 9,500,000 has been reported. On hydrolysis it yields galactose, rhamnose and galacturonic acid. Gum karaya occurs as a partially acetylated derivative. The acid number has been found to vary from 13.4 to 22.7. The variation in acid number is influenced not only by the source of the sample but also by its age. The gum has a peculiar property of splitting off free acetic acid and this loss is loosely correlated with the particle size. Trimethylamine has also been identified in the hydrolysis products. Gum karaya contains 12% to 14% moisture; less than 1% acid insoluble ash.

pH: The pH of a 1% gum karaya solution is 4.6. If small amounts of alkali are added to change the pH to 7 or 8, the gum tends to have a buffering effect and will gradually reduce the pH again to the acid side.

Preservatives: Gum karaya sols and jellies require preservatives since they are subject to microbial attack. They are easily preserved with a mixture of 0.15% methyl and 0.02% propyl paraben as well as glycerin and propylene glycol.

Compatibility: Gum karaya is compatible with other plant hydrocolloids as well as proteins and carbohydrates. There is apparently an incompatibility of gum karaya gels with pyrilamine maleate, a strong hydrotrope and antihistaminic. Electrolytes also cause a viscosity drop as well as excessive acid. Alkalies cause the gels to become stringy.

Uses: Gum karaya has many varied uses, its primary quality being its cold water swelling and suspending properties. Since gum karaya sols lose viscosity upon standing, they should be used relatively rapidly after preparation.

Pharmaceuticals: bulk laxatives because of natural water absorption and bulking. *Denture Powders:* because of its bland taste and firmness of adhesion. *Cosmetics:* hair wave lotion and hair set concentrates.

Irish moss

Identity and Origin: Irish moss is the dried, sun-bleached, red seaweed *Chondrus Crispus or Gigartina mamillosa.* It is a perennial algae widespread in distribution and commonly found on rocky and broken seashores. In seawater it assumes many colors; forking and multiple branches are typical. When dry, the plants tend to assume a horny appearance. Commercial sources are Nova Scotia, Portugal and France. The greater part of the moss gathered is scraped from the rocks with special long handled rakes which fishermen manipulate from dories.

carrageen is a yellowish-white powder used in combination with other plant hydrocolloids or sugar or other ingredients depending on individual requirements.

Solubility: Irish moss purified forms a sol in hot water; only in the presence of salts does it form a gel. Actually, as normally extracted, Irish moss tends to form a gel.

Irish moss is insoluble in alcohol and other organic solvents. If those liquids, miscible with water, are introduced slowly the solution will not cause precipitation up to 30% concentration.

The hydration of Irish moss is also affected by salts; the potassium cation tending to form gels, whereas sodium is least effective in this respect.

Viscosity: The viscosity of an 1¼% powdered Irish moss sol at 25° is approximately 150 cps.

The addition of salts rapidly changes the viscosity of the sol. However, the characteristics of the gel rather than the sol becomes more important when Irish moss is used in the normal way, and for this reaction to take place the presence of salts is essential.

Irish moss extracts

The previous description refers to Irish moss purified by essentially mechanical means. Of more interest to the pharmaceutical and cosmetic industry has been the extract of Irish moss prepared by hot water extraction of the seaweed and subsequent drying of the extract.

Chemical Characteristics: Irish moss and Irish moss extract molecules have the characteristic ability to bind water and to combine with certain proteins (particularly milk proteins) at low concentrations (on the order of 0.1%) and are useful for suspending, gelling, and viscosity building properties.

Irish moss is normally obtained as a mixed salt of a sulfate ester of a complex of D-galactose units. There are apparently two types of these strongly negatively charged polymers: a kappa and a lambda carrageenan molecule. The lambda molecule has some anhydro-D-galactose units in the polymer. Molecular weight averages 200,000. Normal cations present are sodium, potassium, calcium and magnesium. Although Irish moss extracts hydrate slowly in cold water they are only completely soluble in hot water (65° C for 30 Minutes). When the sodium cation is essentially the only one present, the product tends to form a high viscosity type sol, while the potassium ion tends to cause a gel. The amount of gelling can thus be controlled, and specific standardized products are available.

pH: The pH of a 1% Irish moss extract sol will be in the range of 7 to 9. These sols and gels are extremely stable under neutral conditions, relatively less stable under alkaline conditions, and are broken down under acidic conditions.

Preservatives: In common with all natural, water-soluble gums, Irish moss products are subject to microbial decomposition. The most effective preservatives are the parabens which are permissible up to 0.2%.

Compatibility: Irish moss is compatible with other

plant hydrocolloids and milk products. The sensitivity of Irish moss to inorganic salts is an important characteristic, not shared by other hydrocolloids such as agar. It is also compatible with most organic detergents (alkyl sulfonates). Gel formation requires salts and the degree of gel formation can be closely controlled by the potassium cation.

Strong oxidizing agents cause depolymerization Coagulation by chemical reaction forming insoluble carrageenates is another possibility.

Gel Formation: Irish moss forms gels of various strengths under varying conditions of temperature concentration, and salt concentration. The gels formed are thermally reversible; they melt at higher temperatures than those at which they are formed.

The difference between gelling and melting temperatures is constant at all concentrations of potassium chloride. As the concentration of potassium chloride is increased, the gel strength is also increased as is the rigidity of the gel. In some cases there will be a tendency to bleed (synersis).

The addition of certain gums, such as locust bean or guar, will enhance the flexibility of the gel, although with a decrease in transparency.

Uses: Irish moss is another plant hydrocolloid with a wide range of usefulness. It can be used with a host of other types of materials such as sugars, other vegetable gums, proteins, salts, etc. Its main value is as a stabilizer, as an emollient and demulcent but it has some emulsifying actions. *Pharmaceutical* laxative emulsion; inhibition of pepsin in treatment of peptic ulcer. *Cosmetics*: emulsions, creams, tooth pastes—short texture type, hand lotions—for its emollience and lubricity, face masks.

References

The following literature cited includes general reviews of the various natural plant hydrocolloids as well as papers published specific properties or interactions of these hydrocolloids.

1. Mantell, C. L., The Water-Soluble Gums, Reinhold Publishing Co N. Y., N. Y. (1947).
2. Whistler, R. L., editor, Industrial Gums, Academic Press, In New York, N. Y. (1959).
3. Smith F. and Montgomery, R., Chemistry of Plant Gums a Mucilages, A.C.S. Monograph No. 141, Reinhold Publishing New York, N. Y. (1959).
4. Natural Plant Hydrocolloids, A.C.A. Advances in Chemistry Ser No. 11 (1954).
5. Physical Functions of Hydrocolloids (1960) A.C.A. Advances Chemistry Series No. 25.
6. Levy, G., Natural Gums in Cosmetic Formulation, J. Soc. C Chem. 10 395-402 (1959).
7. Stoloff, L., Polysaccharides Hydrocolloids of Commerce Advan in Carbohydrate Chemistry annual review (1958). p. 265.

Gum Tragacanth:

1. Levy, G. et al., Tragacanth Solutions I, J. A. Ph. A., Sc. Ed., 451 (1958).
2. Levy, G. et al., Tragacanth Solutions II The Determination Thickening Capacity and Stability. Drug Standards 26, 153 (19
3. Levy, G. et al., Tragacanth Solutions III, J. A. Ph. A. Sc. Ed., 695 (1958).
4. Eisman, P. C. et al., Influence of Gum Tragacanth on the Bac cidal Activity of Preservatives, J. A. Ph. A. Sc. Ed., 46, 144 (19
5. Taub, A., Meer, W. A. and Clausen, L. W., Conditions for Preservation of Gum Tragacanth Jellies, J. A. Ph. A., Sc. Ed., 235 (1958).

Irish Moss:

1. Graham Horace D., Quantitative Aspects of the Interaction Carrageenan with Cationic Substances I—Interaction with Met ene Blue, Food Research 25, 720-730 (1960).
2. Graham, H. D. & Thomas L. B., Interaction of Carrageenan other Hydrocolloids with Alkaloids I. J. Pharm. Sci. 50, 483 (19

Miscellaneous:

1. Hutchins, H. H. & Singiser, R. E., New Gums for Pharm. Je J. A. Ph. A., Prac Ed. 46, 226 (1955).

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1972 Edition Food
Chemicals Codex

Residue on evaporation. Between 5 percent and 10 percent.
Specific gravity. Between 0.848 and 0.856.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).
Heavy metals (as Pb). Not more than 40 parts per million (0.004 percent).
Lead. Not more than 10 parts per million (0.001 percent).

75

TESTS

Angular rotation. Determine in a 100-mm. tube as directed under *Optical Rotation*, page 939.

Refractive index, page 945. Determine with an Abbé or other refractometer of equal or greater accuracy.

Residue on evaporation. Proceed as directed in the general method, page 899, heating for 5 hours.

Specific gravity. Determine by any reliable method (see page 5).

Arsenic. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 865.

Heavy metals. Prepare and test a 500-mg. sample as directed in *Method II* under the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Lead. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Packaging and storage. Store in full, tight, preferably glass, or tin-lined containers in a cool place protected from light.
Functional use in foods. Flavoring agent.

GUAR GUM

DESCRIPTION

A gum obtained from the ground endosperms of *Cyamopsis tetragonolobus* (L.) Taub. (Fam. *Leguminosae*). It consists chiefly of a high molecular weight hydrocolloidal polysaccharide, composed of galactan and mannan units combined through glycosidic linkages, which may be described chemically as a galactomannan. It is a white to yellowish white, nearly odorless, powder. It is dispersible in either hot or cold water forming a sol, having a pH between 5.4 and 6.4, which may be converted to a gel by the addition of small amounts of sodium borate.

2S3+41

IDENTIFICATION

A. Transfer a 2-gram sample into a 400-ml. beaker, moisten it thoroughly with about 4 ml. of isopropyl alcohol, add with vigorous stirring 200 ml. of cold water, and continue the stirring until the gum is completely and uniformly dispersed. An opalescent, viscous solution is formed.

B. Transfer 100 ml. of the solution prepared in *Identification Test A* into another 400-ml. beaker, heat the mixture in a boiling water bath for about 10 minutes, and then cool to room temperature. No appreciable increase in viscosity is produced (*distinction from locust bean gum*).

SPECIFICATIONS

Galactomannans. Not less than 66.0 percent.

Limits of Impurities

Acid-insoluble matter. Not more than 7 percent.

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Ash (Total). Not more than 1.5 percent.

Heavy metals (as Pb). Not more than 20 parts per million (0.002 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Loss on drying. Not more than 15 percent.

Protein. Not more than 10 percent.

Starch. Passes test.

TESTS

Galactomannans. The difference between the sum of the percentages of *Acid insoluble matter*, *Total ash*, *Loss on drying*, and *Protein* and 100 represents the percent of *Galactomannans*.

Acid-insoluble matter. Transfer 1.5 grams, accurately weighed, into a 250-ml. beaker containing 150 ml. of water and 15 ml. of 1 percent sulfuric acid. Cover the beaker with a watch glass and heat the mixture on a steam bath for 6 hours rubbing down the wall of the beaker frequently with a rubber-tipped stirring rod and replacing any water lost by evaporation. At the end of the 6-hour heating period add about 500 mg. of a suitable filter aid, accurately weighed, and filter through a tared Gooch crucible provided with an asbestos pad. Wash the residue several times with hot water, dry the crucible and its contents at 105° for 3 hours, cool in a desiccator, and weigh. The difference between the weight of the filter aid and that of the residue is the weight of *Acid-insoluble matter*.

Arsenic. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 865.

Ash (Total). Determine as directed in the general method, page 868.

Heavy metals. Prepare and test a 1-gram sample as directed in *Method II* under the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Lead. A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Loss on drying, page 931. Dry at 105° for 5 hours.

Protein. Transfer about 3.5 grams, accurately weighed, into a 500-ml. Kjeldahl flask and proceed as directed under *Nitrogen Determination*, page 937. The percent of nitrogen determined multiplied by 5.7 gives the percent of protein in the sample.

Starch. To a 1 in 10 solution of the gum add a few drops of iodine T.S. No blue color is produced.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Stabilizer; thickener; emulsifier.

GUM GUAIAC

Guaiac Resin

DESCRIPTION

The resin of the wood of *Guajacum officinale* L. or of *Guajacum sanctum* L. (Fam. *Zygophyllaceae*). It occurs as irregular masses enclosing fragments of vegetable tissues, or in large, nearly homogeneous masses, and occasionally in more or less rounded or ovoid tears; externally brownish black to dusky brown, acquiring a greenish color on long exposure, the fractured surface having a glassy luster, the thin pieces being transparent and varying in color from brown to yellowish orange. The powder is moderate yellow brown, becoming olive-brown on exposure to the air. It has a balsamic odor and a slightly acrid taste. Gum guaiac dissolves incompletely but readily in alcohol, in ether, in chloroform, and in solutions of alkalies. It is slightly soluble in carbon disulfide and in benzene.

IDENTIFICATION

A. Add 1 drop of ferric chloride T.S. to 5 ml. of an alcoholic solution of the sample (1 in 100). A blue color is produced which gradually changes to green, finally becoming greenish yellow.

B. A mixture of 5 ml. of an alcoholic solution of the sample (1 in 100) and 5 ml. of water becomes blue upon shaking with 20 mg. of lead peroxide. Filter the solution, and boil a portion of the filtrate. The color disappears but may be restored by the addition of lead peroxide and shaking. Add a few drops of diluted hydrochloric acid T.S. to a second portion of the filtrate. The color is immediately discharged.

[CONTRIBUTION FROM THE DIVISION OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA, ST. PAUL MINNESOTA]

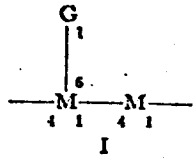
The Constitution of Guar Gum[1]

BY C. M. RAFIQUE AND F. SMITH

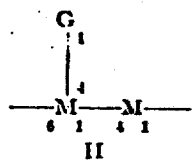
Guar gum extracted from the endosperm of guar seed is a galactomannan polysaccharide in which the ratio of D-mannose to D-galactose is 2:1.[2-5] The occurrence, properties and industrial uses of galactomannans of this type have already been described.[2-11]

This paper is concerned with the constitution of guar gum as revealed by methylation studies. The gum was methylated with methyl sulfate and sodium hydroxide and the separation and identification of the cleavage fragments of the methylated polysaccharide carried out by methods applied previously to the galactomannan of the carob bean.[12,13] Previous methylation studies have shown that 90% of the D-galactose component of the gum occupied terminal positions and that a dimethyl and a trimethyl derivative of mannose were also present in the mixture of cleavage fragments of the methylated polysaccharide.[14]

This work proves that these cleavage fragments consist of approximately equimolecular amounts of 2,3,4,6-tetramethyl-D-galactose, 2,3,6-trimethyl-D-mannose and 2,3-dimethyl-D-mannose. From this it can be deduced that the repeating unit of guar gum is to be represented by any one of the four formulas given below. The preliminary studies of Swanson enabled him to make the tentative suggestion that branched chain structures of this type might be present in the guar gum. All four formulas derive support from the evidence of periodate oxidation which demonstrates that the consumption of four moles of periodate is accompanied by the liberation of one mole of formic acid, a result which is at variance with that of previous workers.[6,8] There is, however, no unique solution to the structural problem although the possibilities may be reduced by recent X-ray evidence, published since this work was completed,



I



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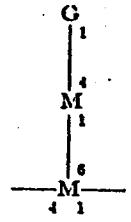
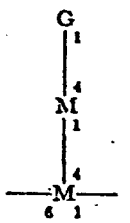


III



IV

G = D-galactose M = D-mannose

which appears to rule out the last two of above four formulas and favors structure I.[15]

While there seems to be little doubt that main structural features discussed above are correct, some additional evidence, as yet unexplain has arisen from the preliminary results of pa partition chromatography[16] of the mixture of ducing methylated sugars derived from the me ylated gum. This evidence shows that in ad tion to the three substances identified, nam 2,3,4,6-tetramethyl-D-galactose, 2,3,6-trimeth D-mannose and 2,3-dimethyl-D-mannose, there small amounts of two others which appear to be p tially methylated derivatives of mannose. W the latter may arise as a result of incomplete me ylation, it is of interest to note that they are s formed from methylated gum which has been s jected to permethylation with pyridinium met iodide.[17] If methylation of the gum is compl and if the additional unidentified fragments do result either from the scission of metho groups[18] or from the condensation of hexose un to give oligosaccharides during methanolysis hydrolysis, then the formation of these two ad tional products will have to be taken into accou when the finer points of molecular structure of gum come to be considered.

It is of interest to note that although the iden fication of the cleavage fragments of methyla polysaccharides is in most cases correctly bas upon the isolation and characterization of cr talline derivatives, it is recognized that the yi of these derivatives is seldom, if ever, quantitat and that a mother liquor is not infrequently d carded. It seems not unlikely therefore th

(1) Paper No. 2537, Scientific Journal Series, Minnesota Agricultural Experiment Station, presented in part before the Division of Sugar Chemistry and Technology at the meeting of the American Chemical Society, San Francisco, 1949.

(2) L. E. Wise and J. W. Appling, *Ind. Eng. Chem., Anal. Ed.*, 16, 28 (1944).

(3) B. W. Rowland, *Chemurgic Digest*, 4, no. 23, 369 (1945).

(4) L. E. Wise, J. W. Green and Ruth C. Rittenhouse, *Tappi*, 32, 335 (1949).

(5) E. Anderson, *Ind. Eng. Chem.*, 41, 2887 (1949).

(6) O. A. Moe, S. E. Miller and Marjorie H. Iwen, THIS JOURNAL, 69, 2621 (1947).

(7) E. Heyne and R. L. Whistler, *ibid.*, 70, 2249 (1948).

(8) R. L. Whistler, Tsiang Kwang Li and W. Dvonch, *ibid.*, 70, 3144 (1948).

(9) R. Hart, *Ind. Eng. Chem., Anal. Ed.*, 2, 329 (1930).

(10) A. L. Williams, *Analyst*, 53, 411 (1928).

(11) J. F. Carson and W. D. Maclay, THIS JOURNAL, 70, 2220 (1948).

(12) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1278 (1948).

(13) F. Smith, THIS JOURNAL, 70, 3249 (1948).

(14) J. Swanson, *ibid.*, 71, 1510 (1949).

(15) K. J. Palmer and M. Ballantyne, *ibid.*, 72, 736 (1950).

(16) *Cf.* E. L. Hirst, L. Hough and J. K. N. Jones, *J. Chem.* 928 (1949).

(17) K. H. Meyer and P. Gürtler, *Helv. Chim. Acta*, 31, (1948).

(18) K. Freudenberg and H. Boppel, *Ber.*, 73, 609 (1940).

mother liquors, such as those encountered in this and other investigations connected with polysaccharides, may well contain compounds of important constitutional significance even though the amounts present are small. However, until the structure and origin of these small amounts of substances have been established, the above four formulas are tentatively advanced to represent the repeating unit of guar gum.

Experimental

The guar gum[19] used in these experiments was a grayish white powder which dissolved in water to give a neutral viscous solution which gave a complex with Fehling solution[20] but did not reduce it even on prolonged boiling. Dilute aqueous solutions of the gum formed a gel when treated with borax.[9,10] When a freshly prepared solution of the gum in water was centrifuged, an insoluble residue amounting to 9.5–11% of the original gum was obtained. This residue which gave positive tests for both proteins and carbohydrates slowly dissolved when kept in prolonged contact with water covered with a layer of toluene. When the insoluble fraction was heated for five hours at 95° with *N* sulfuric acid, a mixture of D-mannose and D-galactose was formed. These were identified by the isolation of methyl-α-D-mannopyranoside and mucic acid respectively.

Hydrolysis of the crude gum with *N* sulfuric acid in the above manner gave a mixture of D-galactose and D-mannose which showed a final value of $[\alpha]^{23}D$ +35° (*c*, 4.4) in acid solution, a value which was found to be in close agreement with that shown by a solution of 2 parts of D-mannose and 1 part of D-galactose in *N* sulfuric acid. The mixture of reducing sugars isolated as above yielded D-mannose phenylhydrazone, m. p. and mixed m. p. 197°, and D-galactose-α-methyl-phenylhydrazone, m. p. and mixed m. p. 173°. Examination of the mixture of sugars on the paper chromatogram using butanol–ethanol–water as the developing solvent[21] confirmed previous findings[4] that D-galactose and D-mannose were the only reducing sugars present.

Fractionation of Guar Gum.—To a solution of the gum in water clarified by centrifugation, methanol was slowly added with stirring to give a series of fibrous precipitates which were washed with methanol, ether and dried *in vacuo* at 60°. The fractions showed $[\alpha]^{23}D$ +60°, +59° and +63°, respectively (*c*, 0.5 in 0.6 *N* NaOH) thus confirming the essential homogeneity of the soluble portion of the gum.[3] Paper partition chromatography of the mixture of sugars produced from these soluble fractions indicated that only D-galactose and D-mannose were present.

Oxidation of Guar Gum with Sodium Periodate.—To a solution of the purified gum (0.2505 g.) in water (150 ml.), 1.53 *N* sodium periodate (30 ml.) was added and the volume quickly adjusted to 250 ml. at room temperature. The mixture was cooled to 5° and kept at this temperature. At suitable intervals an aliquot was removed, treated with excess ethylene glycol, and the formic acid titrated with 0.01 *N* barium hydroxide using methyl red as the indicator.[22] The periodate consumption was determined at the same time on another sample by the usual arsenite method. After forty-eight hours, when the reaction was complete, 0.32 mole of formic acid was produced and 1.36 moles of periodate were consumed per anhydro-hexose unit. In two other experiments, the periodate consumption was 1.25 and 1.3 moles per anhydro-hexose unit (*cf.* refs. 6, 8).

Methylation of Guar Gum.—Since solutions of high concentrations of the gum either in sodium hydroxide or in water could not be stirred efficiently the methylation was conducted as follows. Methyl sulfate (300 ml.) and a solution of the gum (75 g.) in 30% sodium hydroxide (1000 ml.) were added with vigorous stirring to a solution of sodium hydroxide (50 ml.) at such a rate that the reaction mixture always contained an excess of sodium hydroxide. No external cooling was applied. After stirring for two hours the mixture was heated on a boiling water-bath for one hour, cooled, neutralized with dilute sulfuric acid and dialyzed overnight in cellophane bags to remove inorganic salts. Removal of the solvent by distillation *in vacuo* gave the partly methylated gum. Completion of the methylation and isolation of the methylated compound in the manner described in a previous paper[13] yielded a colorless or pale yellow tough glassy solid (yield 50 g.) $[\alpha]^{24}D$ +80.0° in acetone (*c*, 0.4) (found: OCH_3, 45.0). Fractional precipitation of the methyl gum from acetone solution with dry petroleum ether (b. p. 30–60°) in the usual way afforded seven fractions which differed very little from each other in specific rotation ($[\alpha]^{24}_{5461}$ +87° in acetone (*c*, 0.5)) and methoxyl content (45.9%).

Treatment of Methyl Guar Gum with Methyl Iodide in Pyridine.[17]—A specimen of the above methylated gum (1.0 g.) in anhydrous pyridine (50 ml.) was treated with methyl iodide (1.5 ml.) and heated for three hours at 150°. The excess of the pyridine was removed by distillation under reduced pressure and the residue subjected to dialysis for two days against distilled water. The methylated guar gum was recovered from the aqueous solution as described above (found: OCH_3, 44.8).

Methanolysis of Methylated Guar Gum.[12]—When the methylated gum (15.13 g.) was subjected to methanolysis, it reached a constant specific rotation of $[\alpha]^{23}_{5461}$ +91°. Fractional distillation of the mixture of glycosides thus produced gave: *Fraction I* (mainly methyl-2,3,4,6-tetramethyl-D-galactoside) (6.163 g.), b. p. bath temp. 116–120°, 0.06 mm., $n^{20}D$ 1.4498 (OCH_3, 59.3); *fraction II* (a mixture of methyl-2,3,4,6-tetramethyl-D-galactoside and methyl-2,3,6-trimethyl-D-mannoside) (0.818 g.), b. p. (bath temp.) 130°, 0.06 mm., $n^{20}D$ 1.4575, OCH_3, 54.0; *fraction III* (methyl-2,3,6-trimethyl-D-mannoside) (3.260 g.), b. p. (bath temp.) 130–135° (0.06 mm.), $n^{20}D$ 1.4610, OCH_3, 53.0; *fraction IV* (a mixture of methyl-2,3,6-trimethyl-D-mannoside and methyl-2,3-dimethyl-D-mannoside) (0.730 g.), b. p. (bath temp.) 145–155°, 0.06 mm., $n^{20}D$ 1.4708, OCH_3, 41.2; *fraction V* (methyl-2,3-dimethyl-D-mannoside) (4.011 g.), b. p. (bath temp.) 160°, 0.06 mm., $n^{20}D$ 1.4738, OCH_3, 40.2. There was an undistillable residue of 0.65 g. Redistillation of *fraction I* gave *fraction Ia* (5.443 g.) b. p. (bath temp.) 116–118°, 0.05 mm., $n^{20}D$ 1.4480, OCH_3, 59.8. The *residue Ib* (0.466 g.) had $n^{20}D$ 1.4572 and contained OCH_3, 54.8.

On the assumption that the refractive indices of methyl-tetra-, methyl-tri- and methyl-dimethyl-glycoside are $n^{20}D$ 1.4480, 1.4610 and 1.4738, respectively, the weight of methyl-tetramethyl-D-galactoside is 5.579 g. (1.2 mol. prop.) and the weights of methyl-2,3,6-trimethyl and methyl-2,3-dimethyl-D-mannoside are 4.375 g. (1.0 mol. prop.) and 4.570 g. (1.1 mol. prop.), respectively.

In two further experiments, portions of 8.0 g. and 20.0 g. of the methyl gum gave results which indicated that the molecular ratio of the methyl tetramethyl-D-galactoside, methyl-tri- and methyl-dimethyl-D-mannoside were 1.25:1.0:1.2 and 1.4:1.00:1.2, respectively. These results correspond to a composition of 35% D-galactose and 65% D-mannose approximately for guar gum (*cf.* ref. 2).

Identification of 2,3,4,6-Tetramethyl-D-galactose.—Hydrolysis of *fraction Ia* (1.72 g.) with 1 *N* sulfuric acid in the usual way gave 2,3,4,6-tetramethyl-α-D-galactopyranose (1.53 g.) b. p. (bath temp.) 130–135°, 0.1 mm., m. p. and mixed m. p. 70–72°, $[\alpha]^{24}D$ +162°, changing in forty-two hours to +138°, equilibrium value in water (*c*, 0.6); the rotation $[\alpha]^{24}D$ +102° changing to +83° is shown in ethanol and not in water as quoted erroneously by one of us (F. S.) in a previous paper.[13] *Anal.* Calcd. for $C_{10}H_{20}O_6$: OCH_3, 52.5; Found: OCH_3, 52.0.

(19) The authors wish to thank General Mills, Inc. (Minneapolis) for a generous supply of guar gum.

(20) F. May and K. Schulze, *Z. Biol.*, 97, 201 (1936); W. N. Haworth, E. L. Hirst and F. A. Isherwood, *J. Chem. Soc.*, 784 (1937).

(21) S. M. Partridge and R. J. Westhall, *Biochem. J.*, 42, 238 (1948).

(22) T. G. Halsall, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1399 (1947).

Treatment of either the sirup recovered from the mother liquor or the crystalline material with boiling alcoholic aniline in the usual way afforded 2,3,4,6-tetramethyl-D-galactose anilide, m. p. and mixed m. p. 192°; $[\alpha]^{22}D$ −141° in pyridine (*c*, 0.8) (after crystallization from ethanol). *Anal.* Calcd. for $C_{16}H_{25}O_5N$: OCH_3, 40.0; Found: OCH_3, 40.2.

Identification of 2,3,6-Trimethyl-D-mannose.—Hydrolysis of fraction III (3.28 g.) with 2 *N* sulfuric acid at 95° gave 2,3,6-trimethyl-D-mannose as a liquid (2.8 g.), $[\alpha]^{22}D$ +15° in water (*c*, 1.0). *Anal.* Calcd. for $C_9H_{18}O_6$: OCH_3, 41.9; Found: OCH_3, 43.0. The 2,3,6-trimethyl-D-mannose gave the corresponding anilide which, however, proved difficult to purify. Oxidation of this trimethyl sugar with bromine afforded 2,3,6-trimethyl-D-manno-γ-lactone, m. p. and mixed m. p. 79°, $[\alpha]^{18}D$ +60.5° in water (*c*, 0.7).[12,13] *Anal.* Calcd. for $C_9H_{16}O_6$: OCH_3, 42.3; Found: OCH_3, 43.7.

The lactone yielded a phenylhydrazide which crystallized from ethanol as the monohydrate, m. p. 133° (*Anal.* Calcd. for $C_{15}H_{24}O_6N_2 \cdot H_2O$: C, 52.1; H, 7.6; N, 8.1; OCH_3, 26.9; H_2O, 5.2. Found: C, 52.15; H, 7.75; N, 8.3; OCH_3, 27.3; H_2O (loss in wt. at 100°) 5.4. After melting (preferably *in vacuo*) and cooling the anhydrous phenylhydrazide was obtained, m. p. 144° (before or after crystallization from absolute ethanol), $[\alpha]^{20}D$ −16.5° in water (*c*, 0.9). *Anal.* Calcd. for $C_{15}H_{24}O_6N_2$: OCH_3, 28.4; Found: OCH_3, 29.3 (*cf.* refs. 12, 13, 23).

Oxidation of the 2,3,6-trimethyl-D-mannose (1.75 g.) with nitric acid (d. 1.42) produced dimethoxyerythrosuccinic acid which upon esterification with ethereal diazomethane gave methyldimethoxyerythrosuccinate (methyl dimethyl-erythrarate) (0.90 g.) b. p. (bath temp.) 105–110°, 0.05 mm., $n^{26}D$ 1.4320–1.4330, m. p. and mixed m. p. 68° (after crystallization from ether). *Anal.* Calcd. for $C_8H_{14}O_6$: OCH_3, 60.2; Found: OCH_3, 60.0. The ester afforded the corresponding diamide, m. p. and mixed m. p. 257°.[24]

Identification of 2,3-Dimethyl-D-mannose.—One treatment of the methyl-dimethyl glycoside (*fraction V*) with sodium and methyl iodide in liquid ammonia[25] afforded methyl-2,3,4,6-tetramethyl-D-mannoside (found: OCH_3 60.1) which upon hydrolysis yielded 2,3,4,6-tetramethyl-D-mannose, identified as the anilide, m. p. and mixed m. p. 144°.

The methyl dimethylmannoside did not react with sodium periodate (0.05 *N*) at 5° even after six days.

Oxidation of the methyl dimethyl-D-mannoside (0.634 g. *fraction V*) with nitric acid and esterification of the acid so formed as described above for the methyl trimethyl-D-mannoside gave rise to methyl dimethoxyerythrosuccinate (0.35 g.) b. p. (bath temp.) 110°, 0.5 mm., $n^{23}D$ 1.4290–1.4295, m. p. and mixed m. p. 68°. A solution of the crystals in water (*c*, 0.65) was optically inactive. *Anal.* Calcd. for $C_8H_{14}O_6$: C, 46.6; H, 6.2; OCH_3, 60.2; Found: C, 46.7; H, 7.1; OCH_3, 60.1.

Hydrolysis of the glycoside (3.2 g. *fraction V*) with *N* sulfuric acid gave the corresponding 2,3-dimethyl-D-mannose (2.85 g.) $[\alpha]^{27}D$ +22° in water (*c*, 0.7). *Anal.* Calcd. for $C_8H_{16}O_6$: OCH_3, 29.8. Found: OCH_3, 29.7. This dimethyl-D-mannose (55 mg.) afforded an anilide which failed to crystallize. Two treatments of the sirupy dimethyl-D-mannose anilide with silver oxide and methyl iodide yielded the crystalline anilide of 2,3,4,6-tetramethyl-D-mannose, m. p. and mixed m. p., 143°.

Oxidation of the dimethyl-D-mannose (1.0 g.) with bromine in the usual way[13] afforded 2,3-dimethyl-D-mannono-γ-lactone (0.4 g.) as a viscous liquid $[\alpha]^{21}D$ + 64.5° (initial value) in water (*c*, 0.7) changing in fifteen days to +35° (mutarotation incomplete). The lactone crystallized upon nucleation. *Anal.* Calcd. for $C_8H_{14}O_6$: OCH_3, 30.1; Found: OCH_3, 30.2. The lactone gave the phenylhydrazide of 2,3-dimethyl-D-mannonic acid, m. p. and mixed m. p. 156°, $[\alpha]^{22}D$ −24.2° in water (*c*, 1.7).[12,13]

(23) F. Klages, *Ann.*, **509**, 159 (1934); **512**, 185 (1935).

(24) F. Smith, *J. Chem. Soc.*, 371 (1944).

(25) I. E. Muskat, THIS JOURNAL, **56**, 2449 (1934).

Anal. Calcd. for $C_{11}H_{22}O_6N_2$: OCH_3, 21.4; Fou OCH_3, 21.5.

When a solution of the 2,3-dimethyl-D-mannose (0. g.) in 0.2 *N* periodic acid (120 ml.) was kept at 5° for hours, 1.9 moles of periodic acid were consumed per of dimethyl sugar. The periodate and iodate ions precipitated by the addition of barium hydroxide (0.4 and the solution, after filtration, concentrated *in* to about 20 ml. The distillate was treated with a sl excess of 5% alcoholic dimedone and the mixture con trated under reduced pressure to about 25 ml.; this g the dimedone derivative of formaldehyde m. p. and mi m. p. 180° (yield 0.47 g. (after crystallization from wa corresponding to the formation of 0.37 mole of formal hyde per mole of dimethyl sugar). Further experim using sodium periodate as the oxidizing agent instea periodic acid showed that approximately two mole periodate were consumed for each mole of dimethyl su with the formation of 0.9 mole of formic acid. At completion of these periodate oxidations optical acti was almost completely destroyed.

Investigation of the Composition of the Mixture of ducing Methylated Sugars Produced from Methyl Guar Gum by Paper Partition Chromatography.— mixture of methylated glycosides derived from the met ated gum was converted into the corresponding mixtu reducing methylated sugars by hydrolysis with *N* sulf acid in the usual way. Examination of this mixture reducing methylated sugars by paper chromatograp using as the developing solvent the upper layer of a m ture of butanol (40 ml.), ethanol (10 ml.), ammoni hydroxide (1 ml.) and water (49 ml.)[16] furnished the sults given in Table I. Two "spots" with R_G value

TABLE I

R_G[b] VALUES OF SOME METHYLATED SUGARS

This table includes the R_G values of other methyla sugars besides those encountered in this work; these included for reference purposes. The values di slightly from those quoted by Hirst, *et al.*,[16] but thi probably due to a difference in temperature.

	Reducing methylated sugar	R_G values (25°C
1	Mixture from methyl guar gum	0.22,[a] 0.39,[a] 0. 0.87, 0.93
2	2,3,4,6-Tetramethyl-D-galactose	0.93
3	2,3,4,6-Tetramethyl-D-mannose	1.0
4	2,3,6-Trimethyl-D-mannose	0.84
5	2,3,4-Trimethyl-D-mannose	0.86
6	2,3-Dimethyl-D-mannose	0.62
7	4-Methyl-D-mannose	0.40
8	2,3,4,6-Tetramethyl-D-glucose	1.0
9	2,3,6-Trimethyl-D-glucose	0.88
10	2,3-Dimethyl-D-glucose	0.68
11	3-Methyl-D-glucose	0.40

[a] These "spots" showed up only when the sirupy m ture of reducing methylated sugars was applied direc (without dilution) to the starting line of the chroma gram, thus indicating that they were present in sm amounts. [b] R_G = (Distance travelled by the substanc distance travelled by 2,3,4,6-tetramethyl-D-glucose).

0.22 and 0.39 appeared on the chromatogram in addit to the three (R_G, 0.93, 0.87, 0.63) shown, respectively, 2,3,4,6-tetramethyl-D-galactose, 2,3,6-trimethyl-D-m nose, and 2,3-dimethyl-D-mannose. Two unidentif "spots" were also shown by the mixture of reduc methylated sugars obtained from a specimen of met guar gum which had been methylated first by the met sulfate method and then by the pyridinium metho-iod procedure[17] (see above). The unidentified "spots" a appeared in the chromatogram of the reducing methyla sugars obtained from the methyl-trimethyl-D-mannos (*fraction III*) and the methyl-dimethyl-D-mannos (*fraction V*). Complete methylation of each of th

fractions followed by hydrolysis gave a product which showed a single "spot" on the chromatogram corresponding to 2,3,4,6-tetramethyl-D-mannose.

It is possible that the unidentified reducing sugars responsible for the "spots" on the chromatogram with R_G values of 0.22 and 0.39 are produced because the material is not completely methylated but if they are not due to incomplete methylation, the possibility must be borne in mind that the unidentified sugars are of constitutional significance. This problem will be the subject of further study.

Summary

Guar gum is a polysaccharide consisting of pyranose units of D-mannose (2 parts approx.) and D-galactose (1 part approx.) mutually joined by glycosidic bonds. Cleavage of the methylated gum yields approximately equimolecular amounts of 2,3,4,6-tetramethyl-D-galactose, 2,3,6-trimethyl-D-mannose, and 2,3-dimethyl-D-mannose. Each of these fragments has been characterized by the formation of crystalline derivatives. The gum molecule, built of a large number of repeating units containing one D-galactose and two D-mannose residues is highly branched and all side chains are terminated by a D-galactose residue. Four possible structures are postulated for the repeating unit of guar gum.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF SOUTH CAROLINA]

Fluorinated Derivatives of Propane Containing a $-CHF_2$ Group

BY H. W. DAVIS AND A. M. WHALEY

This paper is presented as an extension of previously reported work on fluorine derivatives of propane.[1] Four compounds, each having the nucleus $-\overset{|}{\underset{|}{C}}-CCl_2-CHF_2$ were prepared starting from $CHCl{=}CCl-CHF_2$ (I). Chlorination of this olefin gave successively $CHCl_2-CCl_2-CHF_2$ (II) and $CCl_3-CCl_2-CHF_2$ (III) with no evidence of $CHCl_2-CCl_2-CF_2Cl$ (IV). The fluorination of (III) with antimony trifluoride and catalyst easily formed $CFCl_2-CCl_2-CHF_2$ (V) and $CF_2Cl-CCl_2-CHF_2$ (VI).

These compounds allow for interesting comparisons of the reactivity of the $-CHF_2$ and $-CHCl_2$ groups. In particular, compound (II), $CHCl_2-CCl_2-CHF_2$, since it contains both groups similarly situated, is well suited for such a comparison and has been subjected to two types of reactions: (a) photochlorination and (b) dehydrochlorination. As mentioned above, chlorination of (II) gave only (III) showing that the hydrogen of the $-CHF_2$ group is more resistant to displacement by chlorine than the hydrogen of the $-CHCl_2$ group. Dehydrohalogenation of (II) with alcoholic sodium hydroxide produced $CCl_2{=}CCl-CHF_2$ exclusively, indicating that the hydrogen of the $-CHF_2$ group is also more resistant to attack by alkali. The identity of the dehydrohalogenation product was established by its physical properties and by chlorination to (III).

Further evidence of the contrast in the reactivity of the hydrogen in the two groups is to be had from the following facts. The removal of hydrogen chloride from $CCl_3-CCl_2-CHCl_2$ is accomplished with great ease,[2] whereas the dehydrohalogenation of (III) to form $CCl_3-CCl{=}CF_2$ was not accomplished by the authors in spite of numerous attempts. The above examples lead to the conclusion that the hydrogen in the $-CHF$ group is unusually stable.

It is interesting that $CCl_3-CCl_2-CHF_2$ (m. p. 139.9°), although it contains the smaller fluorine atoms, melts 111° higher than $CCl_3-CCl_2-CHCl_2$.

The structure of (II) is assigned on the basis that it was made by chlorinating $CHCl{=}CCl-CHF_2$ and has the composition of a simple adduct. The structure of (III), m. p. 139.9°, is proved in that it was made by chlorination of (II), which could produce only two pentachlorides, one of which is known to be a liquid.[3]

It has been demonstrated that antimony trifluoride introduces fluorine into a $-CCl_3$ group in preference to a $-CCl_2-$ group[4] and that the boiling point is lowered approximately forty degrees for each chlorine–fluorine replacement in the $-CCl_3$ group.[5] Therefore the structures of compounds (V) and (VI) are assumed to be as given.

Experimental

Chlorine was added to 13.1 moles $CHCl{=}CCl-CHF_2$ (I)[1] (1930 g.) in sunlight until 13.1 moles (930 g.) was absorbed. Part of the material thus obtained was fractionated for pure $CHCl_2-CCl_2-CHF_2$ (II), b. p. 147.6°, $n^{25}D$ 1.4479, d^{20}_4 1.6582. Analysis for chlorine using a Stepanow reduction[6,7] followed by gravimetric determination of AgCl gave 65.20%; calculated, 65.10%.

Four moles (872 g.) of (II) was chlorinated further in sunlight until the gain in weight was about 100 g. The mixture, which had solidified during chlorination, was fractionated to give 430 g. boiling from 170–176°, which was essentially (III), b. p. 175.2°, m. p. 139.9° as determined from a freezing curve. *Anal.* Calcd. for $C_2HF_2Cl_5$: Cl, 70.26. Found: Cl, 70.45.

Fluorination of $CCl_3-CCl_2-CHF_2$ (III).—Antimony trifluoride, 0.34 mole (60.5 g.), and 0.675 mole of (III) (170 g.) was placed in a one-liter round-bottom flask and 0.167

(1) Whaley and Davis, THIS JOURNAL, 70, 1026 (1948).
(2) Prins, *J. prakt. Chem.*, 89, 414 (1914).
(3) Henne and Ladd, THIS JOURNAL, 60, 2491 (1938).
(4) Henne and Renoll, *ibid.*, 61, 2489 (1939).
(5) Henne, "Organic Reactions," Vol. II, Roger Adams, Editor-in-Chief, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 59.
(6) Stepanow, *Ber.*, 39, 4056 (1906).
(7) Cook and Cook, *Ind. Eng. Chem., Anal. Ed.*, 5, 186 (1933).

activities but occurs too late to explain the early metabolic events mentioned in this and previous papers(1,2,4).

Summary. Measurements of incorporation of ^{14}C from differentially labelled glucose and pyruvate into uterine lipid and CO_2 were used to evaluate early metabolic changes produced by estradiol in immature rat uterus. Tissue was incubated with the isotopes *in vitro* after hormone treatment *in vivo*. With pyruvate-3-^{14}C as substrate, substantially more $^{14}CO_2$ is produced than with glucose-1-^{14}C or glucose-6-^{14}C as substrates. Also, $^{14}CO_2$ output from glucose-1-^{14}C is about double that from glucose-6-^{14}C. Estradiol treatment causes a similar increase of ^{14}C incorporation into lipid with either of the glucose substrates or with pyruvate. This suggests that the estradiol effect on lipid synthesis is beyond pyruvate. A differential effect on $^{14}CO_2$ production from glucose and pyruvate, however, locates an estradiol effect between these substrates. Furthermore, the estradiol acceleration of $^{14}CO_2$ production from glucose-1-^{14}C and glucose-6-^{14}C is equal. This suggests that the site of estrogen stimulation of uterine glucose metabolism is prior to the formation of glucose-6-phosphate and therefore implicates the transport step, the phosphorylation step, or both.

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1. Szego, C., Roberts, S., Recent Progr. Hormone Res., 1953, v8, 419.

2. MacLeod, R. M., Hollander, V. P., Fed. Proc., 1961, v20, 199.

3. Bitman, J., Cecil, H. C., Mench, M. L., Wren, T. R., Endocrinology, 1965, v76, 65.

4. Nicolette, J. A., Gorski, J., Arch. Biochem. Biophys., 1964, v107, 279.

5. Cuppy, D., Crevasse, L., Anal. Biochem., 1963, v5, 462.

6. Gorski, J., Nicolette, J. A., Arch. Biochem. Biophys., 1963, v103, 418.

7. Steel, R. G., Torrie, J. H., Principles and Procedures of Statistics, McGraw-Hill Book Co., New York, 1960, p72.

8. Aizawa, Y., Mueller, G. C., J. Biol. Chem., 1961, v236, 381.

9. Barker, K. L., Warren, J. C., Endocrinology, 1966, v78, 1205.

10. Gorski, J., Noteboom, W. D., Nicolette, J. A., J. Cell Comp. Physiol., 1965, v66, 91.

11. Wood, H. G., Katz, J., J. Biol. Chem., 1958, v233, 1279.

12. Singhal, R. L., Valadares, J. R. E., Life Sci., 1966, v5, 1299.

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Effect of Guar Gum and Pectin N. F. on Serum and Liver Lipids Of Cholesterol Fed Rats. (31844)

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Previous studies in our laboratory(1) have demonstrated the hypocholesterolemic effect of 16 mucilaginous polysaccharides in White Leghorn cockerels fed a semi-purified diet supplemented with dietary cholesterol. Because our results on the relative order of activity of the various mucilaginous polysaccharides in the chick did not agree with those of Ershoff and Wells in the rat(2), it appeared of interest to carry out several experiments in rats comparing guar gum and pectin N. F. under similar conditions.

Methods. Two basal diets were employed. The first of these was a casein-sucrose basal diet similar to that employed by Ershoff and Wells(2); the composition of this diet has been previously reported(3). The second diet was powdered Purina Lab Chow. In the casein-sucrose basal diet, test substances (including 1% cholesterol and 10% corn oil) were added at the expense of carbohydrate; in the Purina Lab Chow basal diet, test substances (including 1% cholesterol and 5% corn oil) were added at the expense of the

TABLE I. Effects of Dietary Supplements on Serum and Liver Cholesterol and Liver Total Lipids of Rats Fed a Cholesterol-Supplemented Casein-Sucrose Diet(1) (6 Animals/Group).

		Results at the end of 28 days			
Group	Supplements fed with basal ration*	Body wt (g)†	Serum total cholesterol (mg/100 ml)†	Liver total cholesterol (mg/g)†	Liver total lipid (%)†
1	None	219 ± 4.2	106 ± 3.3	1.81 ± .07	4.50 ± .22
2	1% cholesterol	227 ± 15.0	146 ± 14.6	15.50 ± 1.97	9.75 ± .87
	Idem + following supplements:				
3	5% pectin N. F.	226 ± 4.2	147 ± 6.9	10.63 ± 1.48	9.95 ±1.22
4	10% " "	197 ± 16.6	132 ± 6.3	9.20 ± 1.08	7.87 ± .64
5	5% guar gum	217 ± 2.6	134 ± 8.0	10.12 ± 1.02	8.56 ± .34
6	10% " "	194 ± 5.5	105 ± 12.2	4.53 ± .62	5.82 ± .48

* Pectin N. F. (citrus) obtained from Sunkist Growers, Ontario, Calif.; methoxyl content 10.5% on a moisture-ash-free basis. Guar gum (A-20-D) obtained from Stein, Hall and Co., Inc., New York.
† Mean ± standard error of mean.

diet. Carworth Farms rats (CFE strain),* 42-55 g, were divided into groups of 6, and each animal was housed individually with food and water *ad libitum*. Body weight and food consumption were determined weekly. At the end of 28 days on the experimental diets, final body weight and total food consumption were recorded for each animal, then the rats were anesthetized with sodium pentobarbital, and a 5.0 ml blood sample was taken *via* heart puncture. Livers were excised, blotted to remove excess fluid, weighed and stored in a freezer until analyzed. Total liver lipids were determined by the method of Shipley *et al*(4), and total cholesterol was determined on the serum and liver by a modification of the Leffler procedure(5) adapted for use on a Technicon AutoAnalyzer.

Results. Data on body weight, serum and liver cholesterol and liver lipids produced by feeding a casein-sucrose diet supplemented with 1% cholesterol and 10% corn oil to rats over a 28-day period are summarized in Table I. The feeding of 10% guar gum or 10% pectin N. F. (Groups 4 and 6) appeared to cause a slight decrease in body weight gain; however, these decreases were not statistically significant from the non-cholesterol- or cholesterol-supplemented control groups (Groups 1 and 2) at the 5% probability level.

Small decreases in serum cholesterol were observed when 5% guar gum and 10% pectin N. F. were incorporated into the diet; however these changes were not statistically significant at the 5% probability level (Groups 4 and 5 *vs* Group 2); on the other hand, feeding of 10% guar gum lowered serum cholesterol to the level of the control group unsupplemented with cholesterol (Group 6 *vs* Group 1).

Liver cholesterol (mg/g) was reduced from the cholesterol-supplemented control (Group 2) with all levels of agents tested; however, 10% guar gum produced a significantly greater reduction than 10% pectin N. F. (Group 6 *vs* Group 4, $p \leqq 0.05$).

Liver total lipids were also decreased with 10% pectin N. F. and 5% and 10% guar gum. However, statistical significance was obtained only with the 10% level of guar gum (Group 6 *vs* Group 2, $p \leqq 0.05$).

A similar experiment was carried out in rats in which the casein-sucrose basal diet was replaced with a powdered Purina Lab Chow diet plus 5% corn oil. The data on body weight, serum and liver cholesterol and liver lipids are summarized in Table II.

Body weight gain was decreased in the groups receiving 10% pectin (Group 4) and 5% and 10% guar gum (Groups 5 and 6) compared to the cholesterol-supplemented control (Group 2); a significant reduction was obtained only with 5% guar gum (Group 5, $p \leqq 0.05$).

Small decreases in serum cholesterol levels

* Obtained from Carworth Farms Inc., New City, N. Y.

TABLE II. Effects of Dietary Supplements on Serum and Liver Cholesterol and Liver Total Lipids of Rats Fed a Cholesterol-Supplemented Powdered Purina Lab Chow Diet (6 Animals per Group).

Group	Supplements fed with basal ration*	Results at the end of 28 days: Body wt (g)†	Serum total cholesterol (mg/100 ml)†	Liver total cholesterol (mg/g)†	Liver total lipid (%)†
1	None	212 ± 5.5	96 ± 1.8	1.80 ± .07	4.58 ± .14
2	1% cholesterol	218 ± 5.2	90 ± 3.1	5.76 ± .31	6.03 ± .18
	Idem + following supplements:				
3	5% pectin N. F.	217 ± 2.7	94 ± 5.3	4.60 ± .50‡	5.60 ± .21
4	10% " "	209 ± 6.0	85 ± 3.8	4.28 ± .53	5.85 ± .25
5	5% guar gum	188 ± 3.9	87 ± 8.2	3.10 ± .28‡	5.15 ± .28
6	10% " "	196 ± 9.3	76 ± 6.3	3.58 ± .58	5.60 ± .37

* Pectin N. F. (citrus) obtained from Sunkist Growers, Ontario, Calif.; methoxyl content 10.5% on a moisture-ash-free basis. Guar gum (A-20-D) obtained from Stein, Hall and Co., Inc., New York.

† Mean ± standard error of mean.

‡ 5% guar gum *vs* 5% pectin N. F., $p \leq 0.05$.

were observed with the high levels of pectin N. F. and guar gum (Groups 4 and 6), but these were not statistically significant at the 5% probability level.

Liver sterols were reduced with all levels of the agents tested, but a statistically significant reduction was obtained only with 5% guar gum (Group 2 *vs* Group 5, $p \leq 0.05$). Five per cent guar gum gave a greater reduction in liver sterols than an equal amount of pectin N. F. (Group 3 *vs* Group 5, $p \leq 0.05$).

Minor reductions were observed in total liver lipids of all treated groups compared to the cholesterol-supplemented control (Group 2), but once again only 5% guar gum (Group 5) was significantly different ($p \leq 0.05$).

The results obtained with the powdered Purina Lab Chow basal diet were not as dramatic as those obtained with the casein-sucrose basal diet; however, it should be noted that supplementation of this diet with 1% cholesterol (Group 2) did not cause an increase in serum cholesterol, and the increases in liver cholesterol and liver total lipids over the non-supplemented control (Group 1) were not as great as those observed when the casein-sucrose basal diet was employed (Table I).

Discussion. The administration of guar gum and pectin N. F. to rats decreased liver sterols and liver total lipids induced by the feeding of 1% cholesterol. Our results on the relative order of activity of these agents in the rat do not agree with those of Ershoff and Wells(2), but do agree with our results in the chick(1); that is, guar gum has a greater order of hypocholesterolemic activity than pectin N. F. Ershoff and Wells(2) stated that pectin N. F. with maximal activity was obtained only when the methoxyl content, on a moisture-ash free basis, was relatively high in the range of 10.7%, and that inactive preparations resulted when the methoxyl content was 5% or less. All of our experiments were carried out with pectin N. F. which assayed 10.5% methoxyl and was also obtained from a citrus source. Therefore, it would appear that this parameter does not account for the different relative order of activity observed with pectin N. F. in our experiments in rats.

The casein-sucrose basal diet employed in these experiments differed from that of Ershoff and Wells(2) in the following respects: their complete diet contained more sucrose (10%), casein (4%) and salt mixture (3%) than our diet; furthermore, our diet contained 2% bone ash, 5% calcium gluconate and 8% gelatin which were not present in their diets. These alterations in the composition of the basal diet could possibly account for the differences in our results.

Supplementation of a powdered Purina Lab Chow diet with 1% cholesterol did not cause

the marked increases in serum and liver cholesterol levels and liver lipids of the rat obtained with the casein-sucrose basal diet; although these elevations were much less, guar gum still demonstrated greater hypocholesterolemic activity than pectin N. F. Similar results had been previously observed in our experiments with chickens(1) fed either the casein-sucrose basal diet or a commercial-type diet prepared from a variety of natural foodstuffs from vegetable or animal sources.

Summary. The oral administration of guar gum and pectin N. F. to rats in a casein-sucrose basal diet greatly reduced the elevations in liver sterol and total liver lipids produced by the feeding of 1% cholesterol. Similar results were also obtained in rats when a powdered Purina Lab Chow diet was employed. Guar gum was considerably more active than pectin N. F., which confirms the results of similar experiments previously carried out with chickens.

1. Fahrenbach, M. J., Riccardi, B. A., Grant, W. C., Proc. Soc. Exp. Biol. and Med., 1966, v123, 321.

2. Ershoff, B. H., Wells, A. F., ibid., 1962, v110, 580.

3. Grant, W. C., Fahrenbach, M. J., ibid., 1959, v100, 250.

4. Shipley, R. A., Chudzik, E. B., Arch. Biochem. and Biophys., 1948, v16, 301.

5. Leffler, H. H., Am. J. Clin. Path., 1959, v31, 310.

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Effect of Advancing Age on Thyroid Hormone Secretion Rate of Male and Female Rats.* (31845)

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A number of reports have been presented concerning the depression of thyroid function with advancing age in domestic and laboratory animals(1-14). Recently, a study was reported on the effect of advancing age (from 25 to 115 days) on the thyroid hormone secretion rate (TSR) of female rats(15). It was observed that the TSR of the same rats at 25 days was 1.52 μg L-thyroxine (L-T_4)/100 g bw and gradually declined to a level of 0.88 μg/100 g bw at 115 days.

Levey determined the TSH concentration in the pituitary of rats by a bio-assay from birth to 56 days of age and noted a progressive increase from 13 mμ/mg to over 100 mμ/mg(16). It has been observed that the pituitary and plasma levels of TSH were low at weaning time and gradually increased until 80 or 95 days of age, then showed a slight decline(17). The present investigations were undertaken to study the effect of age on the TSR of male rats from weaning time until they were about 4 months of age, at 30-day intervals since no data were available on the TSR of the male rats of increasing age. The effects of aging upon TSR in female rats were studied from weaning time up to 8 months of age.

Materials and methods. The TSR determination of 36 male and 29 female rats of the Sprague-Dawley-Rolfsmeyer strain, bred in this laboratory and maintained at a temperature of 78 ± 1°F, with 14 hours of light and 10 hours of darkness, was started when they were 30 days of age. The mean body weight of the males was 151 g and of the females was 117 g. In addition to these 2 groups, the TSR was estimated in a total of 118 male rats at 30 days of age. Carrier-free I^{131} was diluted in distilled water to contain 3 μc/ml for the first month and later 10 μc/ml was administered to each rat intraperitoneally.

The determination of TSR of the growing rats was based on a slight modification of the technique described by Grosvenor and

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Improvement of Guar Meal By Enzymes

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A DEPRESSION in the growth of chickens by the inclusion of guar meal in their diets has been reported by Borcher and Ackerson (1950), Sathe and Bose (1962), Vogt and Penner (1963), Vohra and Kratzer (1964a) and Bakshi *et al.* (1964). Vohra and Kratzer (1964a) showed an improvement in the growth of chickens if the raw guar meal was autoclaved in layers of about 2.5 cm. thickness for 30 minutes at 2 atmospheric pressure and used as the source of protein along with fish meal. Improvement of guar meal by heat has also been confirmed by Bakshi *et al.* (1964). Our earlier work has indicated that growth of chickens was of the same order as on diets containing soybean meal if the autoclaved or the commercially available toasted guar meal was reacted with enzymes. The enzymes destroyed the growth-depressing effects of guar gum, a polysaccharide obtained from guar beans (Vohra and Kratzer, 1964b). The use of enzymes for overcoming growth-inhibitory properties of guar meal has also been recommended by Anderson and Warnick (1964). Our unpublished data have indicated that the growth inhibitory properties of guar meals, raw as well as toasted, are further increased by treatment for 48 hours of these meals with slurries containing sprouted guar beans. The sprouted and dried guar beans still retained growth-inhibitory properties for chickens. The average gains in weight for chickens fed soybean meal, raw guar meal, autoclaved guar meal, these guar meals reacted with bean-sprout slurries, and on sprouted guar beans alone were as follows: 344, 144, 217, 87, 149 and 173 gm., respectively.

Whenever an aqueous slurry of an enzyme is used for reacting with guar meal, the reacted product has to be dried to prevent mold development in the diets over the experimental period. This increases the cost of the processing of guar meal. In the present study, a commercially feasible method has been developed for using guar meal in the diets of chickens especially for those countries which grow guar beans and lack soybeans.

EXPERIMENTAL

Day-old, Arbor-Acres broiler type chicks were weighed, banded and distributed into groups of approximate equal average weight, each containing 10 chicks. The chicks were housed in electrically heated battery cages and had access to feed and water at all times. They were weighed twice every week as groups and individually on the last day of the experiment. The feed consumption of each group was recorded over an experimental period in which feed wastage could be minimized.

The composition of the diets is given in Table 1. The lots of guar meal[1] and soybean meal contained about 37% and 50% crude protein, respectively. Two different samples of fish meal were used in these experiments. The enzyme preparations were obtained from commercial sources and their properties are listed below:

[1] Courtesy of General Mills.

TABLE 1.—*Composition of the experimental diets*

Ingredient	Diet 1	Diet 2
	Inclusion, gm./kg.	
Soybean meal, 50% protein	150	—
Guar meal, 37.18% protein	—	214
Fish meal, 65% protein	90	90
Soybean oil	20	20
Milo, ground	633.7	605.7
Alfalfa meal, 20% protein	27	27
Cellulose powder[1]	36	—
$CaHPO_4H_2O$	12	12
$CaCO_3$	15	15
NaCl, iodized	4	4
$MnSO_4H_2O$	0.25	0.25
ZnO	0.05	0.05
Vitamin mix[2]	12	12

[1] Solka Floc, Brown Company, New Hampshire.
[2] Vitamin mix supplies in mgs., niacin, 80; calcium pantothenate, 40; pyridoxine HCl, 20; riboflavin, 10; thiamine HCl, 10; folic acid, 3; menadione, 3; biotin, 0.4; vitamin B_{12}, 0.05; choline chloride (44%), 4550; vitamin A (dry, 10,000 I.U./gm.) 1,000; vitamin D_3 (dry 15,000 I.C.U./gm.) 100; and vitamin E (dry, 44 units/gm.), 750.

Enzyme	*Properties*
Keratinase, crude[3]	Breaks down keratin-like material.
Pectinase[3]	Breaks down pectin-like polysaccharides.
Rhozyme-CL[4]	Lipolytic properties.
Cellulase-36[4]	Attacks cellulose and hemicellulose
Fungal amylase[5]	Alpha-amylase activity.
Diazyme[5]	Converts starch and dextrin to glucose.
Cellulase CE-100[5]	Attacks hemicellulose and gums
Cellulase CE-4000[6]	Depolymerizes cellulosic materials.

The above enzymes had the property of reducing the viscosity of guar gum gel.

The term reacted guar meal implies reacting the toasted guar meal (1284 gm.) with a solution (750 ml.) of 24 gm. Cellulase or Rhozyme-CL or 50–100 ml. Keratinase at 37°C. for about 48 hours and then drying the product at 65°C. in a forced draft oven.

In some experiments, the enzymes were mixed with the feed in a dry form without any adjustments in the composition of the diets. The term "wet" is used in those cases where the guar meal was stirred with an aqueous solution (300 ml.) of the necessary amount of enzyme in a Hobart mixer for ½ hour, left at a temperature of about 25°C. overnight and then mixed with the other ingredients in the diets. No further drying was done and there was some danger of the feed getting moldy.

The data on the growth of chickens in experiment 1 were analyzed for statistical significance by the Students' t test. Multiple range and multiple F tests of Duncan, as described in Experimental Methods for Extension Workers (1963), were used for the analysis of growth of chickens and their feed consumption in experiments 2 to 5.

RESULTS AND DISCUSSION

The average gain in body weight of the chickens over the experimental period and the amount of feed used per gm. increase in body weight (F/G) for the various experiments is given in Table 2. Guar meal (autoclaved or toasted) was improved tremendously by soaking it in water or reacting it with a number of enzymes such as Cellulase CE-100, Rhozyme-CL, and crude Keratinase (experiment 1). The treatment of Cellulase CE-100 treated guar meal with crude Keratinase did not improve it any further (unpublished data). Guar meal reacted with Cellulase-36 was not as useful in this experiment as the guar meal to which dry enzymes Cellulase-36 or Cellulase CE-100 had been added. Both these enzymes when added at a level of 4 gm. per kilo to diets containing autoclaved guar meal gave as good growth of chickens

[3] Courtesy of Merck and Co.
[4] Nutritional Biochemicals Corp., Cleveland 28, Ohio.
[5] Courtesy of Rohm & Haas Co.
[6] Courtesy of Miles Chemical Co.

TABLE 2.—*The gain in body weight of chickens and their feed efficiency (F/G) when fed diets containing soybean meal or guar meal and enzymes*

Treatment	Experiment no. 1		Experiment no. 2*		Experiment no. 3*	
	Gain** gm.	F/G	Gain*** gm.	F/G	Gain*** gm.	F/G
	Durations in days 23	11	21	14	21	14
Soybean control	324[e]	1.38	289[b]	1.57[a]	278[b]	1.55[a]
Guar, toasted	181[a]	2.45	175[a]	2.03[d]	110[a]	2.43[c]
Guar reacted w. Cellulase CE-100, 0.4%	292[cde]	1.64				
Guar reacted w. 50 ml. Keratinase	292[cde]	1.74				
Guar reacted w. 100 ml. Keratinase	281[bcd]	1.70				
Guar treated w. water	242[abc]	1.79				
Guar reacted w. Cellulase-36, 0.4%	219[ab]	—				
Guar reacted w. Rhozyme-Cl, 0.4%	247[bc]	1.69				
Guar+dry Cellulase CE-100, 0.4%	285[cde]	1.74				
Guar+dry Cellulase-36, 0.4%	299	1.72				
Guar+0.3% Cellulase-36			264[b]	1.68[ab]		
Guar+0.4% Cellulase CE-100			255[b]	1.82[c]		
Guar+0.66% Cellulase-36			248[ab]	1.78[bc]		
Guar+50 ml. Keratinase					144[a]	2.17[b]
Guar+100 ml. Keratinase					132[a]	2.20[b]
Guar+Cellulase CE-4000, 0.2%					239[b]	1.79[a]
Guar+Cellulase CE-4000, 0.4%					232[b]	1.82[a]

* Average of 2 groups per treatment.

** The values which do not differ significantly at 1% level according to Students' t test have common letters.

*** The values which do not differ significantly by Duncan's analysis for variance at 1% level have common letters.

as the diet which contained enzyme-reacted guar meal. The growth of chickens fed soybean diets was superior to those on enzyme treated guar meal and this may be partly due to the tremendous difference in the fiber and protein content of these plant protein sources. However, the improvement in growth of chickens by the addition of dry enzymes to diets containing guar meal looked very promising and further trials were conducted to determine the minimum levels of enzymes which would give this response. The feed efficiency (F/G) was also improved by the addition of enzymes.

An increase of enzyme (Cellulase CE-100 and Cellulase-36) level to 4 gm. or 6.6 gm. per kilo diet did not improve the growth of chickens or the feed efficiency any further in experiment 2. Cellulase CE-4000, at levels of 2 or 4 gm. per kilo diet was equally effective in overcoming deleterious properties of guar meal and improving the feed efficiency of the chickens in experiment 3, but an addition of 50 ml. or 100 ml. crude Keratinase was ineffective. Guar meal was not improved by the addition of Diazyme, pectinase, or fungal amylase at levels of 2 gm./kg. diet in experiment 4, Table 3. From the data of experiments 4 and 5, it appears that a minimum level of 1 gm. to 2 gm. per kilo diet of the Cellulase type enzymes was as effective as higher levels in overcoming the growth inhibitory properties of guar meal; and the addition of dry enzymes to the diets was as effective as the wet treatment of guar meal with these enzymes. The feed consumption per gm. gain in body weight was less when enzymes were added to the diets containing guar meal. An addition of 1 gm./kg.

TABLE 3.—*The gain in body weight of chickens and their feed efficiency (F/G) when fed diets containing soybean meal or guar meal and enzymes*

Treatment	Weight gain and feed efficiency over 21 days			
	Experiment no.			
	4*		5*	
	Gain** gm.	F/G**	Gain** gm.	F/G**
Soybean meal	268^{f}	1.83^{a}	253^{abcdef}	1.85^{abc}
Guar, toasted	131^{abc}	2.38^{bcd}	183^{a}	2.18^{bcd}
Guar+Diazyme, dry, 0.2%	121^{a}	2.49^{cd}		
Guar+Pectinase, dry, 0.2%	128^{ab}	2.53^{d}		
Guar+Amylase, dry, 0.2%	135^{abcd}	2.53^{d}		
Guar+Cellulase CE-100, dry, 0.1%	192^{e}	2.07^{ab}		
Guar+Cellulase CE-100, wet, 0.1%	183^{cde}	2.27^{cd}	192^{ab}	2.21^{bcd}
Guar+Cellulase CE-100, wet, 0.05%	183^{cde}	2.18^{abcd}		
Guar+Cellulase CE-100, dry, 0.2%	209^{e}	2.15^{abc}		
Guar+Cellulase CE-100, dry, 0.3%	197^{e}	2.16^{abcd}		
Guar+Cellulase-36, dry, 0.05%	181^{bcde}	2.24^{bcd}		
Guar+Cellulase-36, wet, 0.05%	168^{abcde}	2.27^{bcd}		
Guar+Cellulase CE-100, wet, 0.2%			206^{abcde}	2.00^{abcd}
Guar+Cellulase CE-100, wet, 0.3%			201^{abc}	2.25^{d}
Guar+Cellulase-36, dry, 0.1%			259^{bcdef}	1.82^{ab}
Guar+Cellulase-36, dry, 0.2%			273^{ef}	1.85^{abc}
Guar+Cellulase-36, dry, 0.3%			278^{f}	1.78^{a}
Guar+Cellulase-36, wet, 0.1%			222^{abcdef}	2.97^{abcd}
Guar+Cellulase-36, wet, 0.2%			203^{abcd}	2.17^{abcd}
Guar+Cellulase-36, wet, 0.3%			272^{def}	1.78^{a}
Soybean meal+0.1% Cellulase-36, dry			260^{cdef}	1.89^{abcd}

* Average of 2 groups per treatment.
** The values which do not differ significantly at 1% level have common letters.

diet of Cellulase-36 did not improve the soybean diet, indicating that the response with guar meal diets was due to a constitutent of guar meal.

In general, if guar meal is to be used in chicken diets, these diets should be supplemented with enzymes capable of breaking down hemicellulose and gums. Cellulase-36, Cellulase CE-100 or Cellulase-4000 are such enzymes.

SUMMARY

Diets containing about 20% toasted or autoclaved guar meal along with 0.1 to 0.2% enzymes (Cellulases) which hydrolyze guar gum, caused a marked improvement in the growth of chicks.

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REFERENCES

Anderson, J. O., and R. E. Warnick, 1964. Value of enzyme supplements in rations containing certain legume seed meals or gums. Poultry Sci. 43: 1091–1097.

Bakshi, Y., C. R. Creger and J. R. Couch, 1964. Studies on guar meal. Poultry Sci. 43: 1302.

Borcher, R., and C. W. Ackerson, 1950. The nutritive value of legume seeds. X. Effect of

autoclaving and trypsin inhibitor test for 17 species. J. Nutrition, 41: 339–345.
Sahe, B. S., and S. Bose, 1962. Studies on the utilization of industrial and farm by-products in growing poultry rations. Indian J. Vet. Sci. 12: 74–84.
University of California, Agricultural Extension Service, 1963. Experimental Methods for Extension Workers.
Vogt, H., and W. Penner, 1963. Die Verwendung von Guarmehl im Geflugelmastfutter. Arch. Geflügelk. 27: 43–47.
Vohra, P., and F. H. Kratzer, 1964a. The use of guar meal in chicken rations. Poultry Sci. 43: 502–503.
Vohra, P., and F. H. Kratzer, 1964b. Growth inhibitory effect of certain polysaccharides for chickens. Poultry Sci. 43: 1164–1170.

A Chromatographic Investigation of Egg Yolk for the Presence of Steroid Estrogens[1,2]

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AS long as 1925 Fellner (1925) reported that the injection of a lipoid extract from 30 hen eggs into an ovariectomized rabbit evoked an estrogenic response of the same order as that evoked by a similar extract prepared from two human placentae. Allen *et al.* (1924) had previously reported negative results for a rat uterus bioassay of an extract of 18 egg yolks but did report positive results for similar extracts of ovarian follicles. Kopeć and Greenwood (1929) injected five yolks from new-laid eggs into a Brown Leghorn poulard over a period of 20 days. Thirty-eight days after the last injection of egg yolk they observed that feathers regenerated from areas plucked just before the first injection were displaying marked female characteristics and they explained this 'by attributing to the yolk the specific function of the reproductive organ'.

Glimm and Wadehn (1926), using a rabbit uterus assay and a rat vaginal smear assay, could not detect any feminine hormone in hens' eggs.

Serono and Montezemolo (1936) could detect no estrogenic activity in unincubated eggs but did detect activity after the first few days of incubation, this activity increasing to a maximum on the 10th to 12th day of incubation. Riboulleau (1938a) performed bioassays on a crude unsaponifiable fraction of egg yolk lipids. He calibrated his bioassay against 'folliculin' (presumably estradiol) and stated his results in terms of the activity of a given weight of folliculin. Tests on infertile eggs yielded negative results. Tests on fertile eggs indicated an activity equivalent to that of 1.75 to 2 micrograms folliculin per g. yolk, equivalent to approximately 30 micrograms folliculin per yolk of a fertile egg before incubation. Injection of yolk extracts into Leghorn capons produced plumage changes in the direction of the fe-

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Guar Gum, Locust Bean Gum, and Others

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Locust bean gum and guar gum are neutral galactomannan polysaccharides of growing industrial importance. Other less important galactomannans are obtained from legume seeds—for example, alfalfa, clover, and fenugreek. The typical branched structure of these polysaccharides gives them their significant colloidal attributes with important properties for thickeners, solution stabilizers, adhesives, sizes for paper and textiles, and many other applications including food and pharmaceutical applications.

Locust bean gum and guar gum are two important polysaccharide gums of commerce. The importance of these gums stems from the fact that they are hydrophilic colloids which swell greatly in water to produce solutions or dispersions of high viscosity and are capable of acting as stabilizers for suspensions. Their dispersions in pure water do not set to gels on cooling but retain their viscous natures. All of these significant properties result from the structural nature of the gums, which are neutral polysaccharides of large molecular weight containing numerous short branches. As on hydrolysis the two sugars D-galactose and D-mannose are obtained, the gums are termed galactomannans.

Gum Sources and Manufacture

Both locust bean gum and guar gum are produced from seed endosperms from the plant family *Leguminosae*, for which they serve as food reserves. Similar gums may be derived from seeds of most, but not all, legume seeds. The endosperm contents of a few plant seeds which contain galactomannans are shown in Table I. Although galactomannans are often found in seeds of forage crops, the specific nature of only the polysaccharides from alfalfa and clover seeds has been investigated (*3, 8, 9*). Fenugreek seed (*5, 6*) galactomannan also has been examined briefly. Each of the investigations has been of preliminary nature.

The principal commercial source of galactomannans, at present, is locust bean seed. Carob trees *(Ceratonia siliqua)* are cultivated widely in southern Europe, on Mediterranean islands, and in northern Africa.

This tree and the gum from its seeds have been known to man since ancient times. In commercial processing the seeds are removed from their pods and the seed coat is milled off. The remaining endosperm may be ground and marketed as such, or it may be heated, placed in boiling water to disperse the gum, and filtered through screens and cloth, and the crude polysaccharide isolated by evaporation of the solution and final drying on trays in hot air or on hot rolls. The product, usually called locust bean gum, is also known as St. John's bread, swine's bread, gum Gatto, gum Hevo, Jandagum, Lakoe gum, Luposol, Rubigum, and Tragon.

A promising domestic source of galactomannan is being developed commercially from the annual guar crop (*13, 14*). This legume, resembling in appearance the soybean plant, is native to India where it is grown extensively as a cattle feed. During World War II it was grown in the United States for its gum, and the demand for this product continues. At present guar is grown in southern Texas and Arizona, and much valuable agronomic work has been done on this crop by the Arizona Experiment Station. The Experiment Station at Purdue also has recognized the commercial value of guar and has made some attempt to adapt it to growth in more northern latitudes.

Table I. Estimated Endosperm Content of Some Leguminous Seeds[a]

	Endosperm, %
Acacia farnesiana (sweet acacia)	10
Astragalus canadensis (Canada milk-vetch)	8
Baryxylum inerme (sogabark peltophorum)	30
Caesalpinia cacalaco (brazilwood)	30
Cassia absus (senna)	55
Cassia marilandica (wild senna)	50
Cassia occidentalis (coffee senna)	55
Cassia tora (sickle senna)	25
Cercidium torreyanum (palo verde)	20
Ceretonia siliqua (carob)	50
Crotalaria intermedia (rattlebox)	8
Cyamopsis tetragonolobus (guar)	50
Cytisus scoparius (Scotch broom)	15
Daubentonia drummondii (drummond rattlebox)	10
Desmodium canadense (tickclover)	2
Gleditsia triacanthos (honey locust)	30
Gymnocladus dioica (Kentucky coffee)	15
Lespedeza sericea (Chinese lespedeza)	2
Lotus corniculatus (birdsfoot trefoil)	2
Melilotus alba (white sweet clover)	8
Prosopis juliflora (mesquite)	15
Trigonella foenum-graecum (fenugreek)	15
Trifolium hybridum (alsike)	8
Trifolium pratense (red clover)	8
Trifolium repens (white clover)	8

[a] In part after Anderson (2).

In commercial guar varieties the beans occur in pods along the vertical stem of the plant much as in the manner of the soybean plant. Seeds are harvested with standard grain combines after proper adjustment of reel height and speed. In the commercial processing of guar the outer seed coat is removed by passing the seeds rapidly through a flame which slightly scorches the coat and permits it to be removed by a scouring or pearling operation. The approximate composition of the guar flour is shown in Table II.

Table II. Composition of Guar Flour

Component	%
Nitrogen	0.67
Phosphorus	0.06
Ash	1.07
Water-soluble polysaccharide	86.50
Water-insoluble fraction	7.75
Alcohol-soluble (Soxhlet)	1.50

As is evident, the principal component is a water-soluble polysaccharide. Fractionation of this polysaccharide by various means proves that it is composed principally of a single galactomannan (7). For example, when a 1% aqueous suspension is fractionated by the addition of ethanol in small increments, most of the polysaccharide precipitates in a narrow range of alcohol concentration.

Most galactomannans produce on hydrolysis D-mannose and D-galactose in ratios varying from 2:1 to 4:1. Locust bean gum produces about 80% D-mannose and 20% D-galactose. Guar gum produces 65% D-mannose and 35% D-galactose. Average compositions of different galactomannans are shown in Table III.

Molecular Structure

The chemical structures of several galactomannans are fairly well known. This is true of locust bean gum, guar gum, alfalfa gum, clover gum, and fenugreek gum. Perhaps the most fully established structure is that of the guar gum, guaran. The guaran structure, like that of locust bean gum, has been shown to consist of a linear chain of D-mannose units linked together by β-(1→4) glycosidic linkages and having on certain D-mannose units a single D-galactose unit joined by an α-(1→6) glycosidic linkage. The properties of guaran are such as to suggest that, on the average, al-

ome Leguminous Seeds[a]

Endosperm, %
10
8
30
30
55
50
55
25
20
50
8
50
15
10
2
30
15
2
2
8
15
15
8
8
8

in pods along the vertical stem plant. Seeds are harvested with of reel height and speed. In the is removed by passing the seeds he coat and permits it to be re- approximate composition of the

uar Flour

%
0.67
0.06
1.07
86.60
7.75
1.50

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ternate D-mannose units bear a D-galactose unit. Thus, a section of the guaran molecule might be depicted as shown in Figure 1.

Proof that the molecule consists of a long chain of such segments has been obtained from numerous experiments. Initially, it was found that on hydrolysis the molecule produces twice as much D-mannose as D-galactose (*7*). Evidence of numerous branches is suggested from the measurement of the large amount of formic acid produced during quantitative periodate oxidation (*18*). Stress-strain measurements on films of guaran triacetate (*12*) as well as x-ray investigations on films of crude guaran (*10*) reveal that the molecules are highly anisodimensional. The general linear character of the molecule is also demonstrated by the observation that films of guaran triacetate are pliable and are approximately as strong as those of cellulose acetate. They may be stretched extensively without developing crystallinity detectable by x-rays. Consequently the branches must be very short in length.

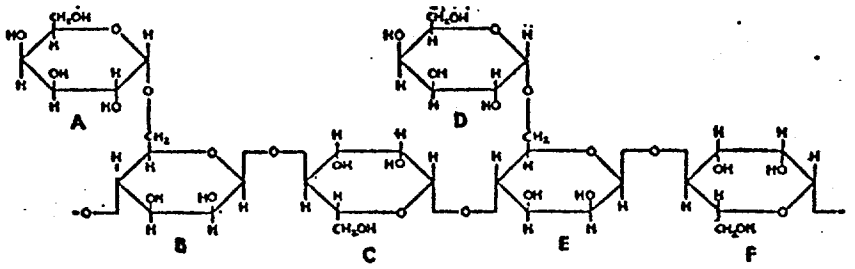

Figure 1. Portion of Guaran Molecule

Methylation of the polysaccharide with subsequent hydrolysis and separation of products leads to the isolation (*1*) of 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-mannose, and 2,3-di-*O*-methyl-D-mannose. These results, confirmed by others (*11*), show that D-galactopyranose units occur as nonreducing terminal units. Since the branches must be short, the most likely structure of guaran, based on evidence to this point, is that shown.

Further evidence for this structure and indication for the anomeric configurations have been obtained by fragmentation of the guaran molecule using partial acid hydrolysis in one case and enzymatic hydrolysis in another. By these processes there are obtained in crystalline form the definitive fragments 4-*O*-β-D-mannopyranosyl-β-D-mannopyranose (*15, 20*), *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-β-D-mannopyranose (*19*), 6-*O*-α-D-galactopyranosyl-β-D-mannopyranose (*15*), and the trisaccharide *O*-α-D-galactopyranosyl-(1→6)-*O*-β-D-mannopyranosyl-(1→4)-β-D-mannopyranose (*16*). The mannobiose and mannotriose could be derived from any two- or three-unit combinations of B, C, E, or F in the guaran structure. The galactomannose disaccharide could be derived from the units A, B or D, E in the indicated structure, while the galactomannose trisaccharide could be derived from the units A, B, C or D, E, F in the guaran structure. The trisaccharide fragment

Table III. Proximate Composition of Some Galactomannans from Legume Seeds[a]

Name of Seed	*Anhydromannose, %*	*Anhydrogalactose, %*
Caesalpinia spinosa (tara)	71	26
Caesalpinia cacalaco (huizache)	69	28
Ceratonia siliqua (carob)	80-86	20-14
Cercidium torreyanum (palo verde)	78	22
Delonix regia (flame tree)	79	19
Cyamopsis tetragonolobus (guar)	64	36
Gleditsia triacanthos (honey locust)	71	26
Gymnocladus dioica (Kentucky coffee)	71	26
Desmanthus illinoensis (prairie-mimosa)	70	26
Indigofera hirsuta (indigo)	72	23
Cassia leptocarpa (senna)	65	21
Crotalaria intermedia (rattlebox)	64	28
Crotalaria juncea (rattlebox)	60	..
Crotalaria striata (rattlebox)	60	..

[a] In part after Anderson (*1*).

theoretically derivable from the combination of units C, D, E has not been found. Nevertheless, the isolation, in crystalline form, of the four fragments listed above, when considered in the light of previous information, is very strong indication that the guaran molecule consists of a linear chain of D-mannopyranose units which are uniformly linked in β-(1→4) fashion and that, on the average, every other D-mannose unit bears a side chain consisting of one D-galactopyranose unit joined by an α-(1→6) linkage. Thus it is apparent that the gum molecules are extremely long and narrow but have difficulty in approaching each other in the uniform manner required for crystallization. It is very likely that it is this comblike structure of the guaran molecule which permits the polysaccharide to display its peculiar and important colloidal properties. The high molecular weight (*4*) of the molecule, on the order of 220,000, is an important contributing characteristic, but it alone is insufficient to explain the colloidal characteristics of galactomannan solutions. The protruding D-galactose units on the D-mannose chain allow the molecule to become highly hydrated by associating itself with a large envelope of water molecules. Complete association between guaran molecules over an extensive portion of their chains so as to produce an aggregating particle which would bring about precipitation is prevented by the protruding D-galactose units which tend to fend off one molecule from another or at least produce such irregularities that extensive interchain association cannot take place. Thus, disperse molecules remain stable and a highly viscous solution results.

Most other galactomannans, and particularly locust bean gum, possess molecular structures similar to guaran; hence their important hydrophilic and colloidal properties are also understandable.

Applications

Locust bean gum and guar gum are advantageously employed in salad dressings, ice cream mixes, bakery products, and other foods. Because of their very strong hydrophilic character they are excellent additives for paper manufacture. Small amounts added to the beaters act as a valuable aid in the hydration of paper pulp by decreasing considerably the time required to hydrate the cellulose fibers to the point where they can form satisfactory paper sheets. Furthermore, by adhering closely to the wood fibers the gums cause the finished sheets to be smoother, to have a higher fold resistance, and to produce an improved wet strength. Mixed with starch, the gums produce textile sizes of improved value. They are superior textile finishes. As thickening agents for textile printing pastes, locust bean and guar gums are unsurpassed. They have proved themselves valuable agents in oil well drilling muds and many other applications. Their derivatives such as the triacetates have properties worth investigating for plastics and coatings.

In commercial practice it is often desirable to bring about controlled modification of gum properties by the use of enzymes. It has been found that germinated guar seeds contain large amounts of an enzyme capable of splitting galactomannans (*17*). Germinated guar seeds, therefore, might represent a source of a commercial enzyme to be used in the controlled modification of galactomannans.

Commercial experience has indicated that these neutral polysaccharides of locust bean gum and guar gum possess so many useful properties that demand for the gums should grow rapidly and they should increase greatly in individual importance.

Literature Cited

(1) Ahmed, Z. F., and Whistler, R. L., *J. Am. Chem. Soc.*, 72, 2524 (1950).
(2) Anderson, E., *Ind. Eng. Chem.*, 41, 2887 (1949).
(3) Andrews, P., Hough, L., and Jones, J. K. N., *J. Am. Chem. Soc.*, 74, 4029 (1952).
(4) Boggs, A. D., master's thesis, Purdue University, 1949.
(5) Bourquelot, E., and Hérissey, H., *J. Pharm. Chem.*, 11, 589 (1900).
(6) Dorud, K. M., *Biochem. J.*, 26, 255 (1932).
(7) Heyne, E., and Whistler, R. L., *J. Am. Chem. Soc.*, 70, 2249 (1948).
(8) Hirst, E. L., Jones, J. K. N., and Walder, W. O., *J. Chem. Soc.*, 1947, 1443.
(9) Muntz, M. A., *Compt. rend.*, 94, 453 (1882).
(10) Palmer, K. L., and Ballantyne, M., *J. Am. Chem. Soc.*, 72, 736 (1950).

(11) Rafique, C. M., and Smith, F., *Ibid.*, 72, 4634 (1950).
(12) Smart, C. L., and Whistler, R. L., *J. Polymer Sci.*, 4, 87 (1949).
(13) Whistler, R. L., *Chem. Inds.*, 62, 60 (1948).
(14) Whistler, R. L., *Implement and Tractor*, 63, 44 (1948).
(15) Whistler, R. L., and Durso, D. F., *J. Am. Chem. Soc.*, 73, 4189 (1951).
(16) *Ibid.*, 74, 5140 (1952).
(17) Whistler, R. L., Eoff, W. H., and Doty, D. M., *Ibid.*, 72, 4938 (1950).
(18) Whistler, R. L., Li, T. K., and Dvonch, W., *Ibid.*, 70, 3144 (1948).
(19) Whistler, R. L., and Smith, C. G., *Ibid.*, 74, 3795 (1952).
(20) Whistler, R. L., and Stein, J. Z., *Ibid.*, 73, 4187 (1951).

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DISCUSSION

Question. To what extent do galactomannans hydrolyze at room temperature?

Dr. Whistler. A neutral solution of these gums will not undergo hydrolysis. To effect degradation, the presence of either hydrogen ions or enzymes is required. In the presence of acid, a partially controlled hydrolysis can be produced, in which the D-galactose units seem to be preferentially split at a higher rate than the main chain of D-mannose units. In the presence of a proper β-galactosidase the D-galactose side-chain units should be preferentially removed without damage to the principal chain of mannose units. In effect, conversion of a galactomannan to a mannan would take place.

What is the evidence of the regularity of the galactomannan chain?

Evidence of a regular galactomannan chain constructed of such units as I have shown is based upon the known composition of the molecule and the isolation upon hydrolysis of the various expected fragments in relatively high yields. Should the arrangement of the chain be irregular, we would expect to obtain these fragments in relatively different amounts. The physical properties of the molecule also tend to indicate that the molecules are long and relatively regular in configuration.

Can locust bean gum and guar gum be used interchangeably? Do they have similar properties?

These two gums have very similar properties in solution. Commercial gums differ, depending upon their source, and there is some evidence that guar gum produces a somewhat more viscous solution than locust bean gum. On the other hand, some locust bean gum and guar gum preparations are equivalent in viscosity.

Is guar gum being produced commercially at the present time, and, if so, how much does it sell for?

General Mills is again producing guar in the Southwest, principally in Texas. It is grown by farmers in alternate years as a secondary crop. I am not certain about the present price of guar flour, but I think it is in the neighborhood of 35 cents per pound.

What is the viscosity of a 4% solution?

I do not remember the exact figures. In general, guar solutions are approximately four times as viscous as solutions of cornstarch at equal concentrations.

How do you measure viscosity?

It depends upon the purpose for which the viscosity is needed. In our own laboratories, where we are concerned primarily with a fundamental characterization of the gum, we measure viscosity in 1*N* potassium hydroxide solution with guar concentrations varying from 0.5 to 1%. From such measurements we can also calculate limiting intrinsic viscosity.

Would these gums have any advantage over synthetic cellulose gums?

The value of a gum depends upon the particular application to which it is put. I suppose you have in mind comparing galactomannan gums with methylcellulose

and other cellulose derivatives. So far as I know there is now no important competition between cellulose derivatives and galactomannan gums. Each has properties which give it special industrial importance.

Can a film be cast from unacetylated gum?

Yes, films can be cast which are clear and pliable, and can be stretched to high degrees. Upon stretching, films undergo crystallization which can be detected by x-rays, and in this way they behave like films prepared from other large linear molecules and resemble cellulose films. X-ray analyses of stretched guar films have been made by Palmer and Ballantyne.

In what pH range can one use locust bean gum for mucilage purposes?

I do not know.

Does the viscosity change with pH?

I have never made such a study. My guess is that the viscosity does vary and that it would have a strong pH dependence.

Has the alpha linkage of the galactose units been established?

I believe that it has been established from two standpoints. First, a galactosylmannose disaccharide on hydrolysis changes in rotation so as to suggest the presence of an alpha linkage. Secondly, the galactosyl units are split from the disaccharide and in some instances from the polysaccharide by means of a proved α-galactosidase.

Is the hydration of guar gum a true hydration or is it similar to cellulose swollen in water?

Cellulose does not swell very greatly in water; it hydrates. For example, cellulose pulp hydrates in paper beaters. The question depends upon what one means by swelling. By hydration one generally means that a hydrophylic molecule becomes associated with an atmosphere of water molecules that are held in a semifixed state and that interpolysaccharide secondary bonds become fewer as a consequence. In other words, the polysaccharide secondary forces are taken up mainly by water molecules. To this extent guar gum and cellulose hydrate in the same way. Guar gum hydrates much more extensively than cellulose; consequently its molecules may become separated so as to form a suspension.

What is the name of the enzyme from germinated guar seed?

It has no name, because it is a complex mixture of enzymes. It contains a mixture of one galactosidase and one mannosidase and perhaps more. We have made an attempt in our laboratory to separate the galactosidase activity from the mannosidase activity, but have not been able to do so in a clear-cut fashion.

Is this enzyme mixture the one you used to modify the chain?

Yes, it is obtained from germinated guar seeds and was a crude guaranase mixture. The crude mixture was capable of bringing about a rapid and extensive modification of the galactomannan.

Do polysaccharide gels become water-resistant after stretching?

I have never made such an examination. I would think that the water resistance so produced would not be extremely great.